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Zebrafish models of human motor neuron diseases: Advantages and limitations

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ABSTRACT

Motor neuron diseases (MNDs) are an etiologically heterogeneous group of disorders of neurodegenerative origin, which result in degeneration of lower (LMNs) and/or upper motor neurons (UMNs). Neurodegenerative MNDs include pure hereditary spastic paraplegia (HSP), which involves specific degeneration of UMNs, leading to progressive spasticity of the lower limbs. In contrast, spinal muscular atrophy (SMA) involves the specific degeneration of LMNs, with symmetrical muscle weakness and atrophy. Amyotrophic lateral sclerosis (ALS), the most common adult-onset MND, is characterized by the degeneration of both UMNs and LMNs, leading to progressive muscle weakness, atrophy, and spasticity. A review of the comparative neuroanatomy of the human and zebrafish motor systems showed that, while the zebrafish was a homologous model for LMN disorders, such as SMA, it was only partially relevant in the case of UMN disorders, due to the absence of corticospinal and rubrospinal tracts in its central nervous system. Even considering the limitation of this model to fully reproduce the human UMN disorders, zebrafish offer an excellent alternative vertebrate model for the molecular and genetic dissection of MND mechanisms. Its advantages include the conservation of genome and physiological processes and applicable *in vivo* tools, including easy imaging, loss or gain of function methods, behavioral tests to examine changes in motor activity, and the ease of simultaneous chemical/drug testing on large numbers of animals. This facilitates the assessment of the environmental origin of MNDs, alone or in combination with genetic traits and putative modifier genes. Positive hits obtained by phenotype-based small-molecule screening using zebrafish may potentially be effective drugs for treatment of human MNDs.

Contents

Abbreviations: AChR, acetylcholine receptor; α-MNs, alpha motor neurons; ALS, amyotrophic lateral sclerosis; AMO, antisense morpholino oligonucleotide; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors; BMP, bone morphogenetic protein; BVVL, Brown-Vialetto-Van Laere syndrome; CaP MNs, caudal primary motor neurons; CMT, Charcot-Marie-Tooth disease; CMT2, axonal form of Charcot-Marie-Tooth disease; CNS, central nervous systems; CST, corticospinal tract; dHMN, distal hereditary motor neuropathy; dpf, days post fertilization; DCSMA, dominant congenital SMA; EMS, embryonic malabsorption syndrome; ER, endoplasmic reticulum; f-ALS, familial amyotrophic lateral sclerosis; FL, Fazio-Londe syndrome; FUS, fused-in-sarcoma; γ-MNs, gamma motor neurons; hpf, hours post fertilization; HSP, hereditary spastic paraplegia; IN, interneurons; LAAHD, lethal arthrogryposis with anterior horn cell disease; LCCS1, lethal congenital contracture syndrome 1; LCST, lateral corticospinal tract; LMC, lateral motor columns; LMNs, lower motor neurons; MiP MNs, middle primary motor neurons; MLF, medial longitudinal fascicle; MNs, motor neurons; MNDs, motor neuron diseases; MMC, medial motor column; NMJ, neuromuscular junction; NMLF, nucleus of the medial longitudinal fasciculus; PLS, primary lateral sclerosis; PMNs, primary motor neurons; PCH, pontocerebellar hypoplasia; RF, reticular formation; RoP MNs, rostral primary motor neurons; s-ALS, sporadic amyotrophic lateral sclerosis; SBMA, spinal and bulbar muscular atrophy; SuC, superior colliculus; SMA, spinal muscular atrophy; SMA-PME, spinal muscular atrophy with progressive myoclonic epilepsy; SMN1, survival of motor neuron 1; SMN2, survival of motor neuron 2; SMNs, secondary motor neurons; SOD1, soluble cooper/zinc superoxide dismutase 1; TARDBP, TAR DNA-binding protein; UMNs, upper motor neurons; VaP, variable primary motor neurons; VCST, ventral corticospinal tract; VEGF, vascular endothelial growth factor; VN, vestibular nuclei; vS, ventrally projecting secondary motor neurons; WT, wild-type.

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1. Introduction

Historically, the term "motor neuron diseases" (MNDs) refers to disorders caused by the degeneration of motor neurons (MNs) located at anterior horn in the spinal cord or at the brainstem motor nuclei, with secondary Wallerian degeneration of motor axons (Desai and Olney, 2009). In fact, MNDs constitute an expanding, heterogeneous group of developmental and neurodegenerative disorders. Recently, substantial progress has been made in deciphering the molecular bases of numerous inherited conditions (Brown and Phil, 2000; Dion et al., 2009; Fink, 2013; Van Damme and Robberecht, 2009). These disorders are anatomically subdivided, depending on whether they involve upper motor neurons (UMNs), *i.e.* premotor neurons with soma located in higher regions of the brain and conveying descending commands for movement, lower motor neurons (LMNs), *i.e.* brain stem cranial motor nuclei and spinal MNs, or both (Brown and Phil, 2000). The UMNs of the central nervous systems (CNS) originate in the motor cortex or brainstem and relay motor information to the LMNs. LMNs are located in the cranial motor nuclei and spinal cord and relay impulses from the UMNs to the muscles at neuromuscular synapses to innervate the skeletal muscles (Pratt et al., 2012). UMN symptoms generally include weakness, speech problems, overactive tendon reflexes, spasticity, Hoffmann signs, Babinski signs, clonus, and inappropriate emotionality. LMN symptoms also include weakness, as well as attenuated reflexes, cramps, twitching, and muscle wasting. Considerable overlaps of clinical, paraclinical, and physiopathological characteristics may be observed between these entities, leading to persistent difficulty in establishing a precise diagnosis in some patients and raising controversies about the classification of these disorders.

Animal models of human MNDs, mostly rodents, but also the fruit fly (*Drosophila melanogaster*) and the nematode worm (*Caenorhabditis elegans*), have been used in attempts to understand the neurobiological basis of MNDs and predict successful treatment strategies (Bebee et al., 2012; Grice et al., 2011; Hirth, 2010; Lanson and Pandey, 2012; McGoldrick et al., 2013; Sleigh et al., 2011). The zebrafish (*Danio rerio*) is a vertebrate species, with a similar overall nervous system organization to humans, and is increasingly used to model human diseases (Seth et al., 2013). It represents a powerful experimental model for studying neurogenetic disorders and MNDs, due to the high conservation of genes implicated in neurodegenerative diseases and physiological processes involved in nervous system morphogenesis and maintenance (Bandmann and Burton, 2010; Becker and Rinkwitz, 2012; Kabashi et al., 2011a; Xi et al., 2011). In view of the current emphasis on high-throughput screening to identify genetic interactions and pharmacological therapies, the zebrafish model has become an attractive alternative to rodents, thanks to lower costs and less time-consuming experiments. This review summarizes current knowledge of the comparative neuroanatomy of human and zebrafish motor systems. A comprehensive overview of current knowledge of human genes and factors involved in MNDs and the use of zebrafish in investigating the biology of human illness are also presented. The validity of the zebrafish model for acquiring knowledge of specific components of MNDs is also discussed.

2. Comparative neuroanatomy of human and zebrafish motor systems

Human motor systems are organized hierarchically in three levels of control: spinal cord, brainstem, and motor cortex. There is a clear division at each level of control between MNs innervating axial muscles and those innervating limb muscles. Zebrafish have at least twenty different neuronal populations in the brain projecting to the spinal cord, mostly located at the brainstem (Becker et al., 1997). However, an important difference between teleost fish species and mammals is that there are no direct telencephalic projections to the spinal cord. Whereas the corticospinal tract (CST) is seen as an adaptation for fine motor control of the limbs in mammals (Iwaniuk and Whishaw, 2000), the brainstem organization of neurons with descending axons is generally more highly conserved among other vertebrates (Goulding, 2009).

2.1. Spinal cord

The human motor nuclei in the spinal cord, the lowest level in the hierarchical organization, are arranged along a medial-lateral axis according to function. The ventral medial motor column (MMC) projects to the axial muscles in the neck and back, the hypaxial motor column projects to the hypaxial muscles in the ventral body wall, and the dorsal lateral motor columns (LMC)

project to the limb muscles (Dasen and Jessell, 2009). This topographic map of the motor nuclei of the spinal cord, arranged in different motor columns innervating different groups of muscles is also present in other mammals, reptiles, and birds, but not in other vertebrates (Fetcho, 1992). Spinal LMNs, innervating muscles fibers, are highly diverse in terms of morphology, connectivity, and functional properties and differ significantly in their response to disease (Kanning et al., 2010). Alpha MNs (α-MNs) innervate extrafusal muscle fibers and drive in-muscle contraction. Gamma MNs (γ-MNs) innervate intrafusal muscle fibers of the muscle spindle and are involved in proprioception. α-MNs, the most abundant class of spinal MNs, can in turn be classified in three subtypes, according to the contractile properties of the motor units that they form with target muscle fibers: slow-twitch, fatigue resistant (S), fast-twitch, fatigue resistant (FR), and fast-twitch, fatigable (FF) (Burke et al., 1973). FF-subtype MNs have large cell bodies and large-diameter, fast-conducting axons, while the S-subtype has the smallest cell bodies and axons. FF MNs seem to be the most sensitive to multiple degenerative disorders, including spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS), and aging.

Zebrafish spinal MNs may be divided into primary and secondary classes, based on when they differentiate and innervate their target musculature (Eisen et al., 1986; Myers et al., 1986). Primary motor neurons (PMNs) are larger, appear during gastrulation, and undergo axogenesis during the first day of development (Lewis and Eisen, 2003). Three to four individual PMNs may be identified in each spinal hemisegment by their cell-body positions, axonal trajectories, and electrical membrane properties (Moreno and Ribera, 2009). Caudal primary motor neurons (CaP MNs) innervate the ventral trunk musculature, middle primary motor neurons (MiP MNs) innervate dorsal trunk musculature, and rostral primary motor neurons (RoP MNs) innervate muscle fibers in between. A fourth class, variable primary motor neurons (VaP MNs), typically undergoes apoptosis by 36 h post fertilization (hpf) (Eisen et al., 1990), innervating muscle fibers located between the territory of rostral and middle PMNs. In a recent paper, Menelaou and McLean (2012) analyzed motor unit properties and recruitment in larval zebrafish, finding two different types of RoP MNs: ventrally- and dorsally-projecting RoPs MNs. Although PMNs exhibit collaterals projecting off the main axons and ramify extensively throughout the deep musculature, some PMNs also present collaterals within the spinal cord (Menelaou and McLean, 2012). PMNs, located in the dorsal part of the motor column, have been classified by their electrical properties as the tonic class of zebrafish spinal MNs, with a high spike threshold, high-frequency tonic spiking, and limited spike-frequency adaptation in response to suprathreshold current injection (Menelaou and McLean, 2012). Secondary motor neurons (SMNs), located more ventrally in the motor column, are smaller, more numerous, appear later, and typically have thinner axons than PMNs. Menelaou and McLean (2012) also categorized SMNs according to the extent of their dorsoventral muscle innervation. Thus, the dorso-ventrally-projecting SMNs, the most dorsally located SMNs, exhibit an innervation field in both the dorsal and ventral musculatures, while the ventrally-projecting secondary motor neurons (vS), with the soma located immediately below the dorso-ventrally-projecting SMNs, have a ventrally-restricted arborization field. These two types of SMNs exhibit more extensive branching off the main axons deep in the muscle tissue, suggesting that they preferentially activate fast muscles. Finally, the dorsally projecting SMNs, with the soma located below the vS, exhibit an innervation field in the dorsal musculature, with the most extensive arborization in fast skeletal muscle. In addition, Menelaou and McLean (2012) demonstrated two additional classes of SMNs with a common anatomical feature: the presence of a major collateral axon running superficially along the intermyotomal boundary. When SMNs were classified on the basis of their electrophysiological properties, two subtypes were reported. On one hand, the chattering class of spinal MNs, with a lower spike threshold than the tonic class and a significant spike frequency adaptation during prolonged subthreshold current injection. On the other hand, the bursting class of spinal MNs, with a lower spike threshold, that produce slow membrane potential changes. Although there were no exclusive correspondences between these two electrophysiological classes of SMNs and any morphological classes of SMNs, bursting MNs tended to be more ventral than chattering MNs.

The somata of zebrafish PMNs and most SMNs are located in the spinal cord motor column. Moreover, MNs located in the ventral most region of the ventral horn innervate the paired fins, and in the case of the pectoral fin MNs their cell bodies are located in segments three through six (Myers, 1985; Thorsen and Hale, 2007). Such teleost fish appendicular MNs do no segregate into a lateral motor column, and abductor an adductor MNs are mingled within ventral MNs (Fig. 1) (Thorsen and Hale, 2007; Murakami and Tanaka, 2011). In fact, although fin MNs present some similarities with SMNs, as a similar size of the soma and thicknes of the axons, they represent a distinct class of spinal MNs (Myers, 1985). Thus, *islet 1* is not expressed in the zebrafish pectoral fin MNs, but is expressed strongly in SMNs (Higashijima et al., 2000). PMNs have not been described in amniotes, thus human α-MNs may be more similar to SMNs (Beattie et al., 1997). No γ-MNs have been described in zebrafish, which do not have intrafusal muscle fibers.

Axial motor structures formed the primitive motor apparatus of vertebrates, consisting of a rostro-caudal series of myomeres

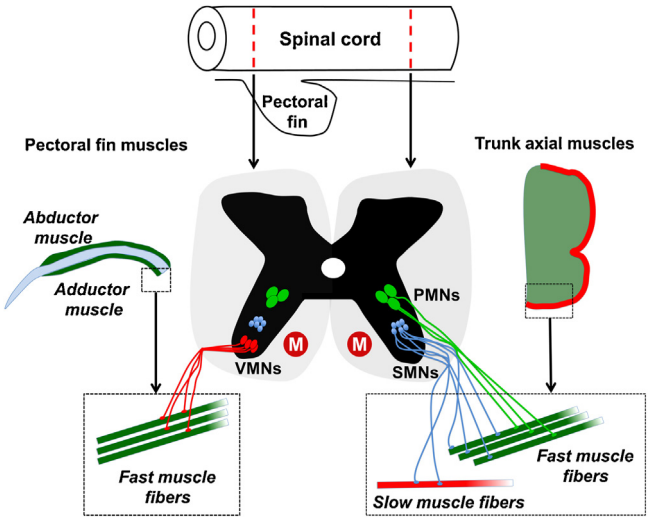

Fig. 1. Schematic summary of the topography of trunk spinal motor neurons (MNs) and the muscles they innervate in a zebrafish larva. For the sake of simplicity, only the innervation of primary (PMNs) and secondary motor neurons (SMNs) on one side is shown. Upper graph is a lateral view at the spinal cord level with anterior part to the left and dorsal part to the top. At the trunk level, zebrafish larvae present a superficial monolayer of slow muscle fibers (red) and in deep portion of the myotome only fast muscle fibers are present (dark green). Larval pectoral fin musculature contains no slow fibers until approximately 7 mm body length. Fast muscle fibers of abductor and adductor muscles and endoskeletal disk of the fin are represented in dark green and blue, respectively. At the mid-trunk level, somata of the PMNs (bright green) are located at the dorsal most region of the motor column of the spinal cord. Axons from PMNs project only on fast muscle fibers (dark green). Somata of SMNs (blue) are smaller, more numerous and are located more ventral than those of PMNs. Axons from these MNs project both to the fast and the slow muscle fibers. Finally, at the pectoral fin level an additional pool of spinal MNs is present (red). These ventral MNs (VMNs) innervating the abductor and adductor muscles at the pectoral fin level have the somata at the most ventral region of the ventral horn. Axon of the Mauthner (M) cell running in the spinal cord is indicated in dark red.

(Fetcho, 1992). This myomeric musculature is also present in most anamniotic vertebrates, including zebrafish. The myomeric musculature in zebrafish is divided into two major types of muscle fibers. Slow fibers, forming a superficial monolayer on the surface of the myotome, are well-equipped for oxidative phosphorylation and more resistant to fatigue. Fast fibers, in the deep portion of the myotome, are more fatigable, as they rely on anaerobic glycolysis for ATP generation (Welsh et al., 2009). Each fast fiber is innervated by a single PMN and up to 4 SMNs, whereas each slow fiber is innervated by several SMNs (Westerfield et al., 1986). Therefore the two groups of fibers can be differentially controlled and perform different functions. Slow fibers are activated during slow sustained swimming, whereas fast fibers only become active during rapid swimming or escape movements (Fetcho, 1992). Electrophysiological evidence indicates that slow fibers are tonic fibers, homologous to the tonic fibers described in the mammalian extra-ocular muscle and are, therefore, not the homologs of mammalian slow twitch muscle, while fast fibers are a twitch-fiber type (Buss and Drapeau, 2002). Molecular and histochemical studies have not made this distinction, simply classifying fibers on the basis of the expression of slow or fast heavy chain myosin isoforms. One question that arises is whether it is appropriate to compare fast, *i.e.* twitch, and slow, *i.e.* tonic, muscle development in zebrafish with fast and slow twitch muscle development in mammals. Moreover, different subtypes of SMNs have been described in the juvenile/adult zebrafish motor column, related to the types of muscle fibers they innervate (Ampatzis et al., 2013; Gabriel et al., 2011). In spite to the above described differences, skeletal muscle fibers in zebrafish share many molecular and histological features with mammalian muscle fibers, including preservation of the components of the dystrophin-associated glycoprotein complex, the excitation-contraction coupling machinery and the contractile apparatus (Gibbs et al., 2013).

2.2. Brainstem

The human brainstem contains many groups of UMNs projecting to the spinal cord through two main systems: the medial and lateral brainstem pathways. These two descending pathways control different groups of spinal neurons and muscles. The ventromedial brainstem descending pathways, phylogenetically the oldest component of the human descending motor systems, consist of the reticulospinal, vestibulospinal, and tectospinal tracts. The fibers arising from the brainstem reticular formation, vestibular complex, and superior colliculus terminate bilaterally in the MMC of the spinal cord, projecting mainly onto interneurons and long propriospinal neurons, but also directly onto some spinal LMNs innervating epaxial muscles in the dorsal body region (Fig. 2A). The medial pathways provide the basic postural control system for head, neck, trunk, and proximal limb movements (Ghez and Krakauer, 2000; Lemon, 2008). The main dorsolateral brainstem descending pathway is the rubrospinal tract, originating in the magnocellular portion of the red nucleus in the midbrain. In humans and rats, the rubrospinal tract descends in the contralateral dorsal part of the spinal cord LMC, projecting onto interneurons controlling LMNs at the cervical level of the LMC (Fig. 3A and B). The lateral pathway is more concerned with controlling more distal limb segments: elbow and wrist (Lemon, 2008; Purves et al., 2001).

Sixteen distinct brainstem nuclei containing about 220 neurons in total, including the reticular formation and vestibular nuclei, as well as the nucleus of the medial longitudinal fascicle, have been found to project to the zebrafish spinal cord (Becker et al., 1997; Gahtan et al., 2002). In larval zebrafish, escape-related activity may be found in 82% of these neurons (Gahtan et al., 2002). The nucleus of the medial longitudinal fascicle contains the most rostral cluster

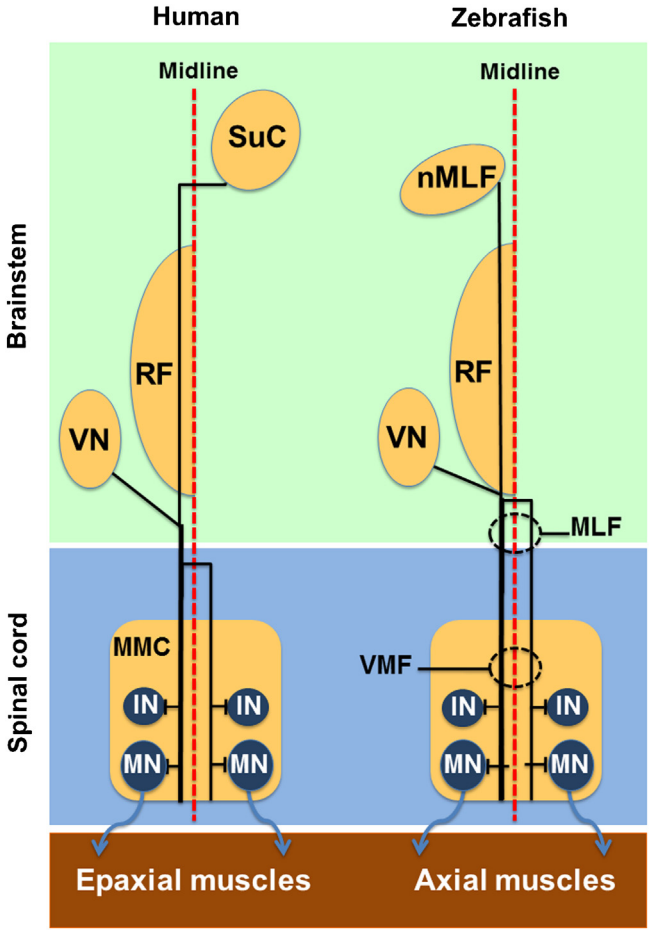

Fig. 2. Schematic representation of the ventromedial brainstem descending pathways in humans and zebrafish. For the sake of simplicity, only the brainstem nuclei and descending pathways on one side are shown. In humans, fibers (black lines) arising from the reticular formation (RF), vestibular nuclei (VN), and superior colliculus (SuC) form the reticulospinal, vestibulospinal, and tectospinal tracts, respectively. These fibers descend bilaterally in the ventral medial motor column (MMC) of the spinal cord, projecting mainly onto interneurons (IN), but also directly into some spinal motor neurons (MNs) innervating epaxial muscles of the dorsal body region. In zebrafish, fibers arising from the RF, VN, and nucleus of the medial longitudinal fasciculus (nMLF) descend from the hindbrain along the medial longitudinal fascicle (MLF), running through the spinal cord as a prominent ventromedial fascicle (VMF). These fibers project at the spinal cord level mainly onto collateral or ipsilateral inhibitory and excitatory IN, as well as directly into some spinal MNs innervating the axial muscles. Although a tectobulbar tract is present (not shown) in zebrafish, no tectospinal tract has been detected.

of zebrafish brainstem neurons, sending ipsilateral projections to the spinal cord (Kimmel et al., 1982). Although a few rubrospinal projections have been found in zebrafish (Becker et al., 1997) (Fig. 3C), the presence of a true rubrospinal tract seems to be related to the presence of limbs or limb-like structures (Donkelaar, 1988). The octavolateral system in nonelectroreceptive teleosts is specialized for detecting auditory, vestibular, and lateral line stimuli. These mechanosensory stimuli are transduced by hair cells in the otolithic organs, *i.e.* the utricle, saccule, and lagena, semicircular canals, *i.e.* anterior, horizontal, and posterior, and lateral line systems (Tomchik and Lu, 2005). In adult zebrafish only neurons from octaval nuclei related to vestibular input, *i.e.* magnocellular octaval nucleus, tangential nucleus, anterior octaval nucleus, and ventrolateral part of descending octaval nucleus, send ipsilateral projections to the spinal cord, reminiscent of the vestibulospinal tract in mammals (Becker et al., 1997; Gahtan and O'Malley, 2003). The presence of a single vestibular nucleus in the young larvae should be noted (Kimmel et al., 1982). The zebrafish

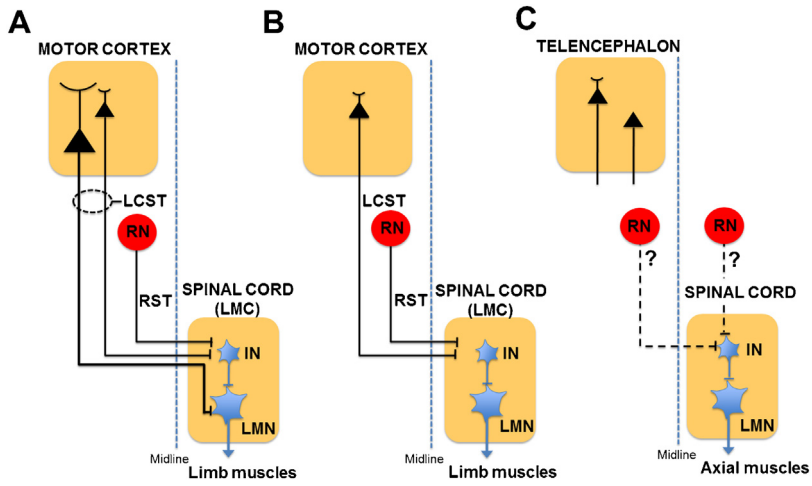

Fig. 3. Comparison of the lateral descending pathways in humans (A), rats (B), and zebrafish (C). For the sake of simplicity, only the descending pathways on one side are shown. In humans, fibers from the lateral corticospinal tract (LCST) reach all the levels of the lateral motor column (LMC) of the spinal cord. Although most of these axons form excitatory synapses with spinal interneurons (IN), some of them project directly onto lower motor neurons (LMN) controlling distal limb muscles. These direct connections between corticospinal and spinal motor neurons are absent in rodents. Zebrafish do not have any direct telencephalic projections onto the spinal cord. Although the red nucleus (RN) is present in all three species, a true rubrospinal tract (RST) descending along the contralateral dorsal part of the spinal cord LMC to project onto spinal interneurons at the cervical level is only present in humans and rats. Dashed lines from the RN to spinal interneurons in C indicate that a few rubrospinal fibers project to the spinal cord in zebrafish, with no information currently available on the contralateral or ipsilateral position of these fibers.

optic tectum receives topographically ordered visual inputs from the retina and then sends motor outputs to the premotor reticulospinal system (Sato et al., 2007) and the nucleus of the medial longitudinal fascicle (NMLF) in the brainstem. Thus, it has been demonstrated that visual prey capture in larval zebrafish is controlled by neurons from the NMLF in series with the tectum (Gahtan et al., 2005). However, no direct tectospinal projections have been identified in bony fish by retrograde labeling (Sánchez-Camacho et al., 2001).

Brainstem reticulospinal neurons are phylogenetically conserved across vertebrates and form a major descending motor control system that receives convergent sensory inputs and sends motor commands to spinal circuitry (Kohashi and Oda, 2008). The zebrafish reticular formation, located in the hindbrain, extends longitudinally through most of the hindbrain tegmentum, from the pretrigeminal isthmic tegmentum to the caudal end of the medulla. This region is the largest source of descending signals to the spinal cord in zebrafish (Becker et al., 1997). There are about fifty hindbrain reticulospinal neurons on each side of the brain, although the best known are the Mauthner neuron and segmental homologs MiD2 and MiD3, which are involved in initiation and directional control over the fast escape behavior (Liu and Fetcho, 1999). In mammals it is believed that the Mauthner neurons and their serial homologs have been replaced by a population of 20–60 "giant neurons" in the caudal pontine reticular-formation in the brainstem (Becker and Becker, 2007). Most of the axons of reticulospinal neurons, both ipsilaterals and contralaterals, descend from the hindbrain along the medial longitudinal fascicle (MLF), running through the spinal cord as a prominent ventromedial fascicle (Metcalfe et al., 1986). Many of the axons running through the MLF show collaterals crossing into the contralateral spinal cord (Gahtan and O'Malley, 2003).

2.3. Motor cortex

The motor areas in the human cerebral cortex provide the ability to organize complex motor acts and execute fine precision movements. The motor cortex UMN axons, descending through the corticobulbar tract, modulate the activity of the cranial nerve (*e.g.* trigeminal, facial, and hypoglossal nuclei) LMNs in the brainstem (Purves et al., 2001). On the other hand, axons of UMNs in the motor cortex, descending through the CST, modulate the activity of the spinal LMNs innervating the trunk and limb muscles, both directly and by modulating the activity of brainstem UMNs. The CST is a major descending pathway for controlling voluntary movements. The corticospinal fibers run together with corticobulbar fibers through the internal capsule to reach the ventral portion of the midbrain. Collaterals of the corticospinal axons reach some UMNs in the brainstem (*e.g.* reticular formation, red nucleus), thus modulating the brainstem descending pathways. In the medulla, they form the medullary pyramid and about 75% of the corticospinal fibers cross the midline in the pyramidal decussation at the junction of the medulla and spinal cord. The crossed axons descend *via* the spinal cord LMC, forming the lateral corticospinal tract (LCST) (Fig. 3), projecting to the cervical and lumbosacral spinal cord, to control the distal limb muscles of the hand and foot (Ghez and Krakauer, 2000). Most of the axons descending through the LCST form excitatory synapses with both excitatory and inhibitory interneurons. In contrast, a small number of cortical UMNs, with axons that pass through the LCST, form excitatory synapses directly on spinal LMNs (Fig. 3A). Most of these monosynaptic connections are with LMNs controlling the hand muscles, but the Betz cells have monosynaptic connections to LMNs controlling the lower limb musculature. Betz cells are large pyramidal cells located in the primate primary motor cortex, contributing approximately 3% of the one million axons in the CST and projecting directly onto spinal LMNs at the lumbosacral level (Patestas and Gartner, 2006). It is generally believed that, during evolution, the ability to perform dexterous hand movements developed in parallel to the establishment of these monosynaptic cortico-motoneuronal connections (Isa et al., 2007). The cortico-motoneuronal system is well developed in humans and Old world monkeys and absent in non-primates (Lemon, 2008; Fig. 3B and C). Interestingly, the pathogenesis of HSP is characterized by distal degeneration of the LCST, primarily in the lumbar region (Deluca et al., 2004), with a significant decrease in the density of both small- and large-diameter axons. Indeed, the Betz cell axons, among the largest in the CNS, are the most affected in HSP.

The uncrossed axons descend *via* the ipsilateral ventral column of the spinal cord, forming the ventral corticospinal tract (VCST).

Table 1
Comparative neuroanatomy of human and zebrafish motor systems.

Feature	Key similarities	Key differences and unknowns
Motor Units		
Spinal motor neurons	✓ Zebrafish SMNs similar to human α-MNs. ✓ Different subtypes of MNs, with some located at specific regions of the spinal cord, innervate axial and limbs/fins muscles.	✓ No γ-MNs present in zebrafish. ✓ PMNs have not been described in human. ✓ Appendicular MNs in teleost fish do no segregate into a lateral motor column.
Skeletal muscle fibers	✓ Preservation of the dystrophin- associated glycoprotein complex components. ✓ Similar excitation-contraction coupling machinery. ✓ Similar contractile apparatus.	✓ It is still not well understood if it is appropriate to compare human and zebrafish fast and slow muscle fibers. ✓ Proprioceptors like the *muscle spindle* are not found in Zebrafish.
Brainstem		
Ventromedial brainstem descending pathway	✓ Fibers from the reticular formation and vestibular nuclei descend from the hindbrain, projecting at the spinal cord level mainly onto interneurons, but also on spinal MNs.	✓ Fibers from the optic tectum, zebrafish equivalent to the human superior colliculus, project on the nucleus of the medial longitudinal fasciculus and the premotor reticulospinal system, but a direct tectospinal tract has not been detected.
Dorsolateral brainstem descending pathway		✓ Although a few rubrospinal fibers project to the spinal cord in zebrafish, a true rubrospinal tract is missing.
Motor cortex		
Corticospinal tract		✓ There are no direct telencephalic projections to the spinal cord, *i.e.* corticospinal tract, in teleost.

Many of these axons contain commissural collaterals crossing into the contralateral MMC of the spinal cord. VCST axons project onto interneurons and LMNs in the cervical and lower thoracic spinal cord, modulating the movement of the neck, upper trunk, and proximal-forelimb shoulder muscles (Ghez and Krakauer, 2000).

The CST is a phylogenetically new system that first appeared in mammals and has developed predominantly in primates (Isa et al., 2007). Thus, there are no direct telencephalic projections to the spinal cord in teleosts (Becker and Becker, 2007) (Fig. 3C). Whereas the CST is seen as an adaptation in mammals for fine motor control of the limbs, the brainstem organization of neurons with descending axons is generally more highly conserved among vertebrates, as described above. The absence of corticospinal UMNs in zebrafish is an important feature to be considered when this model is proposed for studying UMN diseases such as HSP, which involves degeneration of the Betz cell axons. However, it is necessary to consider that cortical UMNs projecting directly onto LMNs at the lumbosacral level, specifically targeted in some human UMNs diseases, such as pure HSP, are absent from not only zebrafish, but also rodent models (Fig. 3B and C). The comparative neuroanatomy of human and zebrafish motor systems is summarized in Table 1.

3. Is the zebrafish relevant for understanding the biology of MNDs in humans?

3.1. The good

3.1.1. Genetic tools

The zebrafish model presents many methodological advantages and extensive collections of useful resources are available (reviewed in Bandmann and Burton, 2010; Rinkwitz et al., 2011; Seth et al., 2013) (zfin.org web site, Bradford et al., 2011). These include tools applicable *in vivo*, like whole-mount imaging, thanks to the optical transparency of zebrafish embryos and early larval stages, methodologies for modulating gene expression, behavioral tests to examine changes in motor activity, and simplified simultaneous chemical/drug testing on large number of animals (for recent reviews see Bandmann and Burton, 2010; Laird and Robberecht, 2011; Rinkwitz et al., 2011). The fully sequenced genome is available (Howe et al., 2013), together with molecular anatomical atlases and collections of transgenic lines expressing fluorescent proteins under neuron-specific promoters (Bradford et al., 2011; Flanagan-Steet et al., 2005; Higashijima et al., 2000; Naumann et al., 2010; Satou et al., 2013; Zelenchuk and Brusé, 2011). Numerous gene knockout lines and their short-term associated phenotypes (Kettleborough et al., 2013; Varshney and Burgess, 2013) are now complementary to antisense morpholino oligonucleotides (AMO) transitory knockdown and other genome editing methods (Schmid and Haass, 2013), like transcription activator-like effector nucleases, *i.e.* TALEN (Bedell et al., 2012; Sander et al., 2011; Zu et al., 2013), the CRISPR/Cas system (Hruscha et al., 2013; Hwang et al., 2013; Jao et al., 2013), and transgenic techniques for conditional gene activation or inactivation (Ni et al., 2012). These genetic manipulation techniques are used to investigate the function of a particular gene in the etiology of developmental and/or neurodegenerative MNDs. For example, it is possible to validate the effect of human mutations in zebrafish with function loss or gain methods, using wild-type (WT) or mutated human gene transcripts and knocking down the zebrafish ortholog using AMO (for details see Kabashi et al., 2010a; see Section 3.3 for comments on the genetic differences between these methodological approaches). However, the potential zebrafish ortholog target gene, if any, must, at least, be checked for sequence similarity, gene expression pattern, and synteny conservation between human and zebrafish. It should be noted that the teleost ancestor experienced a whole-genome duplication event at the base of the teleost radiation (Braasch and Postlethwait, 2012). A subsequent rapid gene loss occurred, with an average duplicate retention rate across all genes in teleost genomes estimated to be at 12–24% (Braasch and Postlethwait, 2012), but at variable rates according to the gene function considered (Schartl et al., 2013). Resulting ohnologs may have retained their initial subfunctions but also acquired new functions, *i.e.* neofunctionalization, and/or duplicated genes may be preserved by partitioning ancestral subfunctions between them, *i.e.* subfunctionalization (Postlethwait et al., 2004). The genetic interaction between duplicated genes must be taken in consideration, as recently illustrated with the ALS-associated *tardbp* genes (Hewamadduma et al., 2013; Schmid et al., 2013).

The functional relationship between an underlying human mutation and a disease may be tested experimentally, provided an ortholog with a conserved function has been identified in the model organism. The zebrafish model in its early developmental stages is very useful for establishing a causal link with highly penetrant mutations caused by loss of function mutations or

complete knockdown of a gene's transcript. This would be applicable to genes involved in MN morphogenesis and, to a lesser extent, maintenance. Experiments using zebrafish in early developmental stages demonstrated the power of this model for studying important MN development regulators (Birely et al., 2005; Biswas et al., 2014; Feldner et al., 2005, 2007; Glinka et al., 2010; Hilario et al., 2009; Hutchinson and Eisen, 2006; Laird et al., 2010; Lotti et al., 2012; Morimura et al., 2013; Simpson et al., 2009; Campbell and Marlow, 2013; Tanaka et al., 2007; Schweitzer et al., 2005; Van Ryswyk et al., 2014), including differential subtype effects (Hutchinson et al., 2007; Pineda et al., 2006; Seredick et al., 2012). For example, KBP is a developmental protein involved in axon structure, outgrowth, and maintenance. Zebrafish *kbp* mutants exhibited impaired neurogenesis and early axonal differentiation, phenomena relevant to the cellular basis of Goldberg-Shprintzen syndrome (Alves et al., 2010; Lyons et al., 2008).

Mutations leading to systemic metabolic defects and/or the generation of proteins toxic to neurons that accumulate over time may lead to neurodegeneration and are likely to be involved in the development of late onset MNDs. The numerous gene knockout lines, generated *via* a knockout allele in every protein coding gene in the zebrafish genome (Dooley et al., 2013; Kettleborough et al., 2013), have the potential to explore long-term phenotypic effects, as recently demonstrated with adult siblings of *mfn2* mutants (Chapman et al., 2013).

Unlike mammals, zebrafish have the ability to regenerate their motor circuitry after spinal lesions (Becker and Becker, 2007; Reimer et al., 2008, 2013), have more extensive neurogenic areas in the adult brain (Kizil et al., 2012), and is permissive for regeneration (Fetcho, 2007; Gemberling et al., 2013). However, it is unable to repair MNDs induced by genetic or environmental factors, as demonstrated by numerous examples (see Section 5).

3.1.2. Behavioral genetics

In addition to the many genetic tools available, behavioral genetics are easily accessible in zebrafish. Its stereotyped movement patterns and tactile response abilities may be used to model some of the molecular, electrophysiological, and behavioral aspects of neural diseases (Kalueff et al., 2013; Norton and Bally-Cuif, 2010; Wolman and Granato, 2012). For example, larval complex behaviors are composed of simple behavioral modules that may be associated in a repertoire of stereotyped motor behaviors (Wolman and Granato, 2012). High-speed imaging, combined with software for tracking larval movements at millisecond resolution provides experimenter-independent, high-throughput screening for motor activity deficits (Burgess and Granato, 2007a,b; Fontaine et al., 2008; Mirat et al., 2013). For example, the expression of a human dominant-negative mutation of *KCNC3*, found in spinocerebellar ataxia type 13, reduced K^+ current amplitude and CaP MN excitability and induced locomotor deficits in zebrafish (Issa et al., 2011). Physiological analyses particularly of the NMJ and cellular motor circuits are easily feasible in zebrafish and more difficult to approach in mouse spinal cord. The importance of behavioral assessment in MNDs was also demonstrated in *smn* mutants where larvae with motor axon defects had significant motor behavioral deficits (Hao et al., 2012, 2013).

3.1.3. Screening small molecules with toxic or therapeutic effects on neurons and motor neuron diseases

Zebrafish can be used for high-throughput small-molecule screening and identification of drugs and chemicals (Kaufman et al., 2009; Murphey and Zon, 2006; Rihel and Schier, 2012) able to induce MNDs or rescue an induced MND phenotype, at least in certain aspects of the disease. Whole organism, *in vivo* bioassays may be performed, usually by waterborne exposure: a library of small molecules is arrayed in multiwell plates containing zebrafish embryos or larvae. It should be pointed out that using chemical libraries that already contain Food and Drug Administration/European Medicines Agency-approved drugs facilitates the rapid translation of hits from zebrafish chemical screening to clinical trials (Tamplin et al., 2012).

The neurotoxic effect of some drugs, chemicals, and environmental toxicants on the development of zebrafish spinal MNs has been demonstrated. It is easy to analyze the effect of chemicals on spinal cord MN axonal sprouting and developmental patterns using transgenic lines with specific promoter-driven expression of fluorescent proteins or whole-mount immunodetection methods with PMN or SMN markers, and correlate them with motor behavior impairments. Fig. 4 summarizes several abnormalities in the stereotypic pattern of the spinal MN axonal projections in zebrafish larvae exposed to caffeine and sodium benzoate. Nicotine, cadmium, ethanol, metam-sodium, and chlorpyrifos-oxon have also been reported to have neurotoxic effects on spinal MNs (Kanungo et al., 2011; Menelaou and Svoboda, 2009; Raldúa et al., 2012; Sylvain et al., 2010; Yang et al., 2011). Moreover, caffeine and sodium benzoate also induce different defects at neuromuscular junction (NMJ) level (Chen et al., 2008; Tsay et al., 2007). Zebrafish represent a suitable model for high-throughput screening of a library of chemicals to identify those that specifically impair LMNs. This identification is clearly useful, not only from the point of view of human risk assessment, but also for the potential development of toxic models of LMN diseases. Similarities in the axonal phenotypes after MND-related gene manipulations and those observed after exposure to some toxicants are striking and offer an opportunity for understanding how genetic factors interact with exposure to environmental toxicants and drugs.

Furthermore, one alternative to *in vitro* target-based drug discovery is research guided by phenotype in the context of a whole organism. Thus, animal models of a disease may be used as tools for therapeutic discovery, provided that they faithfully reproduce the disease in question. Phenotype-based approaches may be used to find compounds that modify the disease phenotype, regardless of the specific molecular targets. Zebrafish offer a vertebrate model well-suited to very large-scale, phenotype-based, small-molecule screening to identify chemical suppressors of disease phenotypes (Lieschke and Currie, 2007; Zon and Peterson, 2005). The first zebrafish screening for suppression of a disease phenotype was performed in an aortic coarctation model. The zebrafish mutation gridlock, affecting *hey2*, disrupts aortic blood flow in a physiological manner akin to aortic coarctation in humans (Peterson et al., 2004). Screening 5000 small molecules led to the identification of two structurally related compounds that completely reversed the gridlock phenotype and restored normal circulation. These compounds induced up-regulation of the expression of vascular endothelial growth factor (VEGF) and further experiments demonstrated that the activation of the VEGF pathway was sufficient to rescue the gridlock phenotype. This first study demonstrated the utility of zebrafish pharmacological screening for understanding disease processes and, potentially, discovering new therapies. Since the original gridlock screen, many human diseases have been modeled in zebrafish and screening has been performed to search for suppressors of the disease phenotypes (Lessman, 2011; Peal et al., 2010).

The development of zebrafish models for studying various MNDs, such as HSP, SMA, and ALS, opens up the possibility of using these models for phenotype-driven chemical genetic screens and finding chemical suppressors of genetic phenotypes. Axonotrophic compounds that significantly suppressed motor axon defects in a zebrafish model of SMA were identified after AMO-mediated reductions in SMN1 (Gassman et al., 2013; Wishart et al., 2014).

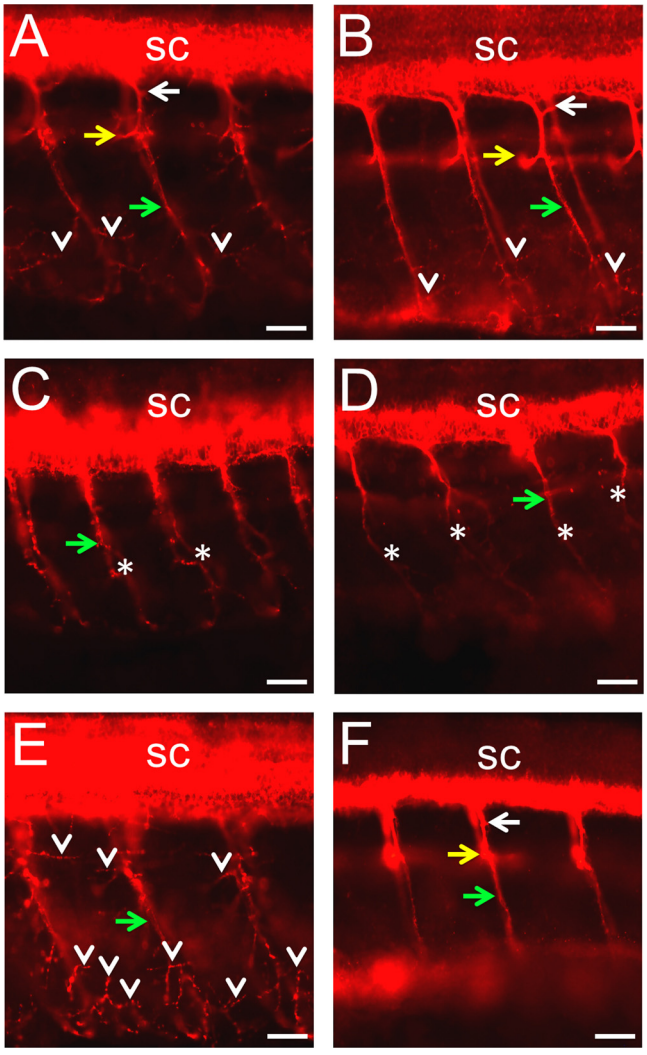

Fig. 4. Neurotoxic effect of caffeine and sodium benzoate on the development of spinal motor neurons in zebrafish. Whole-mount immunofluorescence detection was performed on 57 hpf zebrafish embryos after rearing in fish water (control, A, B) or fish water containing 0.77 mM caffeine (C, D) or 6.9 mM sodium benzoate (E, F) for 31 h. Images are lateral view at the trunk level with anterior part to the right and dorsal part to the top. Antibodies against synaptotagmin (znp1) (A, C, E) and neurolin (zn8) (B, D, F) were used to label the axons emerging from the spinal cord (sc) of primary (PMNs) and secondary motor neurons (SMNs), respectively. (A) In control embryos, rostral (RoP) (yellow arrow), middle (MiP) (white arrow), and caudal (CaP) (green arrow) MN axons innervated fast muscle fibers located in the middle, dorsal, and ventral region of the trunk, respectively. (B) Similarly, axons from rostral-like, middle-like, and caudal-like SMNs are indicated by yellow, white and green arrows, respectively. Moreover, at this developmental stage, some extensions of axonal branching were detected at the CaP and caudal-like SMN axon levels (white arrowheads). (C) Embryos treated with caffeine exhibited some CaP MN axons with abnormal morphology (asterisks) and decreased branching. Moreover, most of the axons of RoP and MiP MNs were absent. (D) Effect of caffeine on SMNs was consistent with the effects on PMNs, with abnormal morphology of the caudal-like SMN axons (asterisks). Embryos also exhibited a complete absence of rostral-like and middle-like SMN axons. (E) Embryos treated with sodium benzoate exhibited axonal CaP MN overbranching (white arrowhead). (F) An early truncation of rostral-and middle-like SMN axons was observed. Scale bar, 50 μm.

However, they had no effect on the survival of smn$^{-/-}$ larvae (Gassman et al., 2013). Methylene blue and structurally related compounds can rescue ALS-like phenotypes associated with mutant TARDBP and FUS through reduction of the endoplasmic reticulum (ER) stress response (Vaccaro et al., 2012, 2013). Dorsomorphin, a small-molecule inhibitor of bone morphogenetic protein (BMP) type I receptors that inhibits BMP signaling, has been shown to rescue *atl1* and *pnpla6* morphant phenotypes, including their spinal motor axon architecture (Fassier et al., 2010; Song et al., 2013). *Epha4* AMO knockdown, as well as the pharmacological inhibition of EPHA4, a receptor in the ephrin axonal repellent system, using 2,5-dimethylpyrrolyl benzoic acid, completely rescued a mutant SOD1-induced motor axonopathy in zebrafish (Van Hoecke et al., 2012). Similarly, genetic knockdown and pharmacological inhibition of Epha4 also attenuated the ALS-induced phenotype in rodents (Van Hoecke et al., 2012), demonstrating the similar mode of action of this modifying pathway on axonal degeneration.

3.2. The bad

AMO knockdown technology is still the most common technique for inhibiting the action of a specific gene or multiple genes simultaneously during zebrafish development, even if DNA-based knockout technologies are currently growing. Researchers must take into account the fact that AMO use has its own limitations. Inhibition is effective only during the first few days of development and potential undesirable off-target effects through p53 activation and neural apoptosis were observed (Bill et al., 2009; Eisen and Smith, 2008; Robu et al., 2007). Off-target mediated effects may have contributed to the observed MN phenotype in *tardbp* morphants (Hewamadduma et al., 2013; Schmid et al., 2013). Therefore, this approach may not be suitable for modeling late onset MNDs, except in cases where late physiological deterioration is only secondary to early-stage neurodevelopmental defects, as suggested in a mouse model of ALS (Martin et al., 2013a,b). In addition, transient AMO models could not be used in some studies such as survival where knockout genetics models are needed.

Another, frequently neglected, limitation is that loss or gain of function of a target gene may result in delayed development, trunk malformations, and decreases in body length and trunk height. Defects in MN abundance and axonal length and branching may not be due to a primary effect on the morphogenesis and maintenance of MNs but a subsequent indirect or side-effect leading to a delay in MN emergence. To cite one example among others, knockdown of the cytochrome c oxidase Vaa subunit using a specific *cox5aa*-AMO showed that, although PMN development was normal at 24 hpf, SMNs, the most similar to mammalian α-MNs, were severely impaired in number and morphogenesis at 48 hpf (Baden et al., 2007). However, the induced phenotype was associated with an embryonic malabsorption syndrome (EMS) (Raldúa et al., 2008), precluding the use of the earlier developmental stages to specifically address the direct effect of a cytochrome c oxidase deficiency at SMN level but an indirect effect may have been mediated *via* nutrient deprivation from the yolk sac. It is necessary to develop genetic models as a way to validate the quicker, but sometimes difficult to use, transient AMO models. As an example, the motor axon defects in the *smn* AMO model (McWhorter et al., 2003) were later recapitulated in a maternal:zygotic-*smn* mutant (Hao et al., 2013). This is an important result as it showed that this is not an off target effect or a secondary defect due to aberrant body morphology. The notion of secondary defects due to trunk malformations was also addressed directly in the context of SMA (McWhorter et al., 2008). Knockdown of *gemin2* caused motor axon defects (Winkler et al., 2005). By performing blastula transplants between *gemin2* AMO treated and WT embryos, it was shown that when *gemin2* is knocked down in MNs, they did not display motor axon defects and when WT MNs were transplanted into *gemin2* morphants, they displayed defects. Thus, the motor axon defects seen in *gemin2* AMO animals were due to defects in body morphology and trunk development.

3.3. The ugly

Human neurological disorders may be modeled in animals using standardized procedures that recreate specific pathogenic events and their behavioral outcomes, but only if there is an evolutionary conservation of the biological processes involved. Comparative neuroanatomy between zebrafish and human central and peripheral nervous systems revealed a similar overall organization, including cell types and neuron structure acquired from their last common ancestor, even if the CNS is considerably smaller in zebrafish than in humans. However, some structural differences must be taken into account when using the former as a model for the latter (Leung et al., 2013; Mueller and Wullimann, 2005; Panula et al., 2010; Rinkwitz et al., 2011; Ronneberger et al., 2012; Wilson et al., 2012; Wullimann et al., 1996). As previously pointed out (Section 2) (Table 1), a review of the comparative neuroanatomy of the human and zebrafish motor systems showed that, while the zebrafish represents a homologous model for LMN disorders, such as SMA, it is only a partial model for UMN disorders, due to the absence of corticospinal and rubrospinal tracts in the zebrafish CNS. However, the zebrafish was recently used as a relevant model of length-dependent axonopathy of the corticospinal MNs (Section 5), indicating that some deterioration in the intrinsic properties of MNs are prominent mechanisms underlying HSPs.

There will probably never be an animal model that is a perfect fit with the human condition. It was initially asserted that only non-human primate models were appropriate for testing pathophysiological hypotheses and evaluating new treatments, while other model species were only appropriate for the molecular and genetic exploration of disease mechanisms (Cenci et al., 2002). After over a decade of using zebrafish as a model for genetic disorders, it is now clear that this is not only an ideal model for visualizing development patterns and the associated diseases but also for studying the functional consequences of mutations of the human genes implicated in a broad range of degenerative disorders. However, most of the work published so far used AMO transient knockdown of target genes at early stages in development, rather than knockout lines and long-term phenotypic surveys. It is, therefore, difficult to detect potential direct or indirect interactions between developmental and neurodegeneration processes, *e.g.* EMS (see Section 3.2.), particularly in the context of late onset MNDs. An AMO model is not a genetic mutant and therefore the term "loss of function" used by some authors should be retained only for genetic mutants that have been verified as loss of function alleles. AMOs more closely resemble to haploinsufficiency which may not be modeled in homozygous mutants, which may be too severe, but rather in heterozygotes. The same precaution may be applied for "gain of function" used instead of RNA overexpression. In most neurodegenerative diseases, expression of neurotoxic proteins is a causative factor and the dominant toxic gain of function ideally requires a heterozygous model generated through a genome editing method. It should be also pointed out that some phenotypes elicited by AMO transient knockdown of target genes involved in neurodegeneration could not be observed in stable genetic knockout lines (Schmid and Haass, 2013).

Furthermore, the use of an animal model for identifying potential molecules to treat patients with diseases does not change the fact that there is a wide gap between discovering that a compound is truly effective on an animal model and its suitability for use in human patients. From this standpoint, the zebrafish model should be viewed as an easily accessible, inexpensive, experimental model for screening potential therapeutic molecules that must be validated by further studies in mammals.

4. Properties of human and zebrafish motor neurons and implications for their selective vulnerability to neurodegenerative diseases

Axon degeneration precedes and, sometimes, causes, neuron death in several disorders, including MNDs. Thus, the common pathological feature of HSPs is a progressive "dying-back" degeneration of the longest axons in the CST and posterior columns. Moreover, other MNDs, such as ALS and SMA, involve major synapse and axon losses before the appearance of symptoms, and long before the loss of neurons. For example, in animal models of ALS, axon loss may reach 60% before the onset of symptoms (Coleman and Ribchester, 2004; Fischer et al., 2004). Although axon degeneration is considered a potential therapeutic target for treating this group of neurodegenerative diseases, new approaches are needed to elucidate the underlying mechanisms. Animal models are very useful for replicating cellular correlates of MNDs such as protein aggregates, inclusions, subcellular defects and cell death rather than modeling pathophysiology or behavioral abnormalities that may be species specific.

Recent data indicate that axon degeneration mechanisms are more uniform than previously thought, even where cause and histopathology differ widely, as in the case of Wallerian degeneration and "dying-back" disease. Axons separated from their cell bodies by nerve transection undergo a program of axon death known as Wallerian degeneration. Understanding of the underlying mechanisms of axon degeneration has come mainly from studying the slow Wallerian degeneration mouse (C57BL/Wld^S), a healthy mutant in which Wallerian degeneration is delayed tenfold. A chimeric Wld^S protein identified in this mutant strain inhibits not only Wallerian degeneration but also axon degeneration in several neurodegenerative diseases. For example, in crosses of Wld^S mice with progressive motor neuropathy mice and the model of Charcot-Marie-Tooth disease (CMT) myelin protein zero mutant, Wld^S significantly delayed dying-back axon degeneration, thus providing a mechanistic link to Wallerian degeneration (Coleman, 2005). Nevertheless, not all axon degeneration is delayed by Wld^S. An important exception includes mouse models of MNDs, such as SOD1 transgenic mouse models of ALS (Velde et al., 2004), the proteolipid protein null model of HSP (Edgar et al., 2004), and mouse models of SMA (Kariya et al., 2009).

Interestingly, an *in vivo* zebrafish model of axon degeneration has been proposed (Bhatt et al., 2004; Feng et al., 2010), based on axotomy of the Mauthner cell. The transparency of zebrafish embryos and larvae makes it possible to visualize the soma and axons of the Mauthner cells directly *in vivo*, by either back-filling or single-cell electroporation with fluorescent dyes. The long axon of this myelinated reticulospinal upper MN regenerates poorly after spinal lesion, so this model is relevant for studying both axon degeneration mechanisms and potential therapeutic targets for axonal regeneration. For instance, using this approach, it was found that cyclic AMP induced regeneration of zebrafish Mauthner cell axons *in vivo* (Bhatt et al., 2004). Moreover, it was recently demonstrated that, as in mice, overexpression of Wld^S in the Mauthner cells delayed axon degeneration in live zebrafish (Feng et al., 2010).

The characteristic hallmark of human MNDs is the progressive and highly selective loss of upper and/or lower MNs. But why are human MNs more susceptible than other neurons or cell types to some genetic disorders and toxins? Indeed, MN cells are continuously active, in order to maintain a particular posture or generate the complex discharge patterns required for locomotor movements. These large cells, considering the membrane surface, may have very long axons; indeed, some human UMNs have axons up to one meter long and axoplasm comprises >99% of total cell volume (Blackstone, 2012). This implies a high capacity for axonal

transport maintenance, lipid homeostasis, mitochondrial respiration, and ionic gradients across the neuronal membrane, factors that may be very closely related. An expanding number of proteins have now been identified that mediate the shaping of the ER network and are involved in its interaction with the cytoskeleton and plasma membrane, as well as other dynamic cellular organelles, such as mitochondria (Goyal and Blackstone, 2013; Rowland and Voeltz, 2012). Some of these proteins are mutated in the most common forms of HSPs (Section 5), indicating that proper ER dynamics, specialization, and distribution are particularly important in large, highly polarized cells such as MNs (Goyal and Blackstone, 2013).

Another property of human MNs relevant for their selective degeneration in MNDs is their high vulnerability to disturbance of mitochondrial respiration (Cozzolino and Carri, 2012). Not only do mitochondria provide the main source of energy in the form of ATP, but they are also the center of the pathway that regulates apoptosis and calcium homeostasis. Thus, both *in vitro* and *in vivo*, inhibition of mitochondrial respiration induces selective MN degeneration. Moreover, the disturbance of mitochondrial respiration sensitizes MNs to stimulation by glutamate, thus increasing their vulnerability to degeneration (Lewinski and Keller, 2005).

Although the precise mechanisms leading to selective degeneration of MNs are far from being understood, disruptions of glutamate transmission seem to be involved, together with the unique properties of human MNs, at least, in ALS and excitotoxic disorders of UMNs. These properties include weak buffering of cytosolic Ca^{2+}, the presence of highly Ca^{2+}-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR), lacking the GluR2 unit, a high neurofilament content, and an exceptional vulnerability to mitochondrial function disruption (Lewinski and Keller, 2005; Weiss, 2011). First of all, disruption of intracellular Ca^{2+} homeostasis is thought to play a key role in MN degeneration. Thus, while Ca^{2+}-binding proteins such as calbindin-D28k and parvalbumin are absent from human cortical and spinal MNs, selective targets for MNDs, other infrequently damaged MN populations express markedly higher levels of these Ca^{2+}-binding proteins. Although no specific data on calbinding-D28k in zebrafish is available, this protein is expressed in both primary and secondary spinal MNs in various fish species (Denizot et al., 1988). However, in *Carassius auratus*, a teleost fish species phylogenetically closely related to zebrafish, calbinding-D28k (*calb1*) is not expressed by all the spinal MNs of a similar size and location (Denizot et al., 1988). Moreover, it has been demonstrated that Ca^{2+} signaling is involved in several of the key stages in zebrafish MN development, including differentiation and axogenesis (Ashworth et al., 2001). Thus, due to this specific role during development, the properties of Ca^{2+} buffering may be very different in embryos and adults.

Together with their low cytosolic Ca^{2+} buffering capacity, the second property of human MNs that may explain their particular vulnerability to increased stimulation by glutamate is the fact that they mainly express the highly Ca^{2+}-permeable AMPAR, with low levels or total absence of the GluR2 subunits. Thus, the increased extracellular glutamate levels observed in some MNDs promote much larger increases in intracellular calcium in human MNs than in other types of neurons (Van Damme et al., 2002; Vandenberghe et al., 2000). Expression of *gria2a* and *gria2b*, zebrafish paralogous AMPAR GluR2 subunits, has been found in 72 hpf zebrafish in the area of the spinal cord where MNs are located (Hoppmann et al., 2008), although to the best of our knowledge no functional data are available on the calcium permeability of zebrafish spinal MNs. However, functional data support the presence of GluR2 subunits in zebrafish Mauthner cells between 33 and 48 hpf (Patten and Ali, 2007).

5. Symptoms of motor neuron degeneration disorders and the use of zebrafish models

5.1. Upper and lower motor neuron degeneration: amyotrophic lateral sclerosis

ALS, also known as Charcot's disease or Lou Gehrig's disease, is the most common form of MND, with an incidence rate varying from 0.3 to 2.5/100,000 (Sathasivam, 2010). The occurrence rate increases after the age of 40 years, peaks in the late 1960s and early 1970s, and declines rapidly thereafter (Logroscino et al., 2008). Death occurs in most patients within 2–5 years after diagnosis. Although the disease is mostly sporadic (s-ALS), 5–10% of cases appear to be familial (f-ALS) (Kiernan et al., 2011; Rowland and Shneider, 2001).

ALS is typically characterized by adult-onset degeneration of the UMNs and LMNs. Disease onset usually begins in the limbs, termed spinal onset, but about a quarter of ALS patients have bulbar onset with initial involvement of facial, mouth/jaw and tongue muscles, associated with poorer prognosis (Pratt et al., 2012). A hallmark of ALS is rapid progression, with subsequent spreading to involve other regions, and, over time, most patients will display both spinal and bulbar features, including emotionality, yawning, jaw jerking, tongue twitching, wasting, drooling, and difficulty swallowing. The diagnosis of ALS may be done by exclusion of other syndromes. The revised El Escorial World Federation of Neurology criteria are frequently used to established a diagnosis of ALS (Brooks et al., 2000) but some criticisms have led to Awaji-shima consensus recommendations, which are likely to increase the sensitivity of the criteria by the use of electrodiagnostic findings, without altering their specificity (de Carvalho et al., 2008).

The pathological hallmarks of ALS are the degeneration and loss of MNs with astrocytic gliosis. Intraneuronal inclusions are present in degenerating neurons and glia (Rowland and Shneider, 2001). TAR DNA-binding protein (TARDBP/TDP-43)-positive ubiquitinated cytoplasmic inclusions are observed in almost all cases of sporadic s-ALS and in most cases of f-ALS (Neumann et al., 2006). Fused-in-sarcoma (FUS)-positive inclusions have been identified in patients with ubiquitin-positive, TARDBP-negative with f-ALS caused by mutations in *FUS* (Vance et al., 2009).

The pathophysiological mechanisms underlying the development of ALS seem multifactorial and operate through interrelated molecular and genetic pathways (Kiernan et al., 2011; Robberecht and Philips, 2013). ALS may be an adult manifestation of a developmental disorder of the human motor system and various environmental risk factors have been suggested in s-ALS. Investigation into susceptibility genes involved in s-ALS has had little success to date. However, 15 genes and loci of major effects implicated in f-ALS have been identified in recent years (Table 2). Mutations in *SOD1* coding for the soluble copper/zinc superoxide dismutase 1, *TARDBP* and *FUS* occur in 20% to 30% of f-ALS. Moreover, expanded GGGGCC (G4C2) hexanucleotide repeats have been very recently identified within intron 1 of *C9ORF72*, revealing the major cause of f-ALS (23–47%) and a genetic cause, which may also be implicated in s-ALS (4–21%) (Millecamps et al., 2010, 2012). The pathogenic mechanism, loss or toxic gain of function, associated with hexanucleotide expansion in *C9ORF72* is currently unknown but even the normal function of this evolutionarily conserved gene is elusive (Stepto et al., 2014).

There are now several studies using the zebrafish as an *in vivo* model for investigating the pathogenesis of ALS (Table 2). Knockdown of the zebrafish *c9orf72* ortholog induced axonopathy of the MNs and specific locomotor deficits, rescued by expressing human *C9ORF72* transcripts (Ciura et al., 2013). Pathologically expanded G4C2 RNA transcripts form intranuclear foci, sequester

Table 2
Main genes involved in human ALS determinism, by frequency, and available published data for modeling the pathology in zebrafish.

Gene	Sub-type (OMIM)	Locus	In	Onset	Pathophysiological mechanisms	Freq in f-ALS	Freq in s-ALS	Ze
C9ORF72	FTDALS (#105550)	9p21	AD	A	? (loss of the C9ORF72 encoded protein, RNA-mediated toxicity, toxic dipeptides from repeat-associated non-ATG translation)	20–50%	5–20%	1, 2
SOD1	ALS1 (#105400)	21q22	AD	A	Detoxification enzyme	10–20%	1–5%	3–10
TARDBP	ALS10 (#612069)	1p36	AD	A	RNA binding and exon skipping	5–15%	1–2%	4, 5, 11–16
FUS	ALS6 (#608030)	16q12	AD, AR	A	RNA binding, exon skipping and DNA repair	5%	<1%	4, 14, 17
ANG	ALS9 (#611895)	14q11	AD	A	Neovascularization	2%	1%	NA
PFN1	ALS18 (#614808)	17p13	AD	A	Disruption of cytoskeletal pathways, axon outgrowth	1–2%	Ex.	NA
ALS2	ALS2 (#205100)	2q33	AR	J	GEF signaling	Ex	Ex	18
SETX	ALS4 (#602433)	9q34	AD	J	DNA and RNA metabolism	Ex	Ex	NA
VAPB	ALS8 (#608627)	20q13	AD	A	Vesicular trafficking	Ex	Ex	19
DCTN1	ALS (#105400)	2p13	AD	A	Axonal transport	Ex	Ex	NA
MAPT	ALS-FTD (#600274)	17q21	AD	A	Microtubule assembly and stability	Ex	Ex	NA
VCP	ALS14 (#613954)	9p13	AD	A	Vesicle transport and fusion, degradation by the proteasome	Ex	Ex	NA
OPTN	ALS12 (#613435)	10p13	AD, AR	A	NF-kB/ubiquitin regulation	Ex	Ex	NA
UBQLN2	ALS15 (#300857)	Xp11	XL	A	Ubiquitin-like protein family, degradation by the proteasome	Ex	Ex	NA

Abbreviations: A, adult; AD, autosomal dominant; AR, autosomal recessive; Ex, exceptional; Freq, frequency; In, inheritance; J, juvenile; NA, not available; XL, X-linked; Ze, Zebrafish bibliographic references: [1]Ciura et al., 2013; [2]Lee et al., 2013; [3]Da Costa et al., 2014; [4]Kabashi et al., 2011b; [5]Laird et al., 2010; [6]Lemmens et al., 2007; [7]McGown et al., 2013; [8]Ramesh et al., 2010; [9]Sakowski et al., 2012; [10]Van Hoecke et al., 2012; [11]Kabashi et al., 2010b; [12]Hewamadduma et al., 2013; [13]Schmid et al., 2013; [14]Vaccaro et al., 2012; [15]Armstrong and Drapeau, 2013b; [16]Vaccaro et al., 2013; [17]Armstrong and Drapeau, 2013a; [18]Gros-Louis et al., 2008; [19]Kabashi et al., 2013.

specific RNA-binding proteins, and are potently neurotoxic in zebrafish embryos (Lee et al., 2013). As in mammals, mutations in zebrafish *sod1* have been shown to cause MN loss (Lemmens et al., 2007). Microinjection of mRNA has been shown to cause transient overexpression of mutant SOD1 in developing zebrafish embryos and may confer a toxic-gain-of-function, with abnormal branching and shortening of MN axons (Lemmens et al., 2007; Sakowski et al., 2012). Transgenic zebrafish overexpressing mutant SOD1 displayed the hallmarks of ALS, including loss of MNs, muscle degeneration, loss of neuromuscular connectivity and defective motor performance, and increased stress in the MNs (McGown et al., 2013; Ramesh et al., 2010; Sakowski et al., 2012). A recent study where a mutant SOD1 protein was expressed at a physiological level demonstrated an early altered NMJ morphology, a susceptibility to oxidative stress, and an adult onset motor phenotype that recapitulated the key features of ALS (Da Costa et al., 2014). *Epha4* was first shown to modulate *sod1* severity in zebrafish, and then subsequently shown to correlate with predisposition in humans, emphasizing the usefulness of this zebrafish model. *Epha4* AMO knockdown as well as the pharmacological inhibition of EPHA4 using 2,5-dimethylpyrrolyl benzoic acid completely rescued a mutant SOD1-induced motor axonopathy in zebrafish (Van Hoecke et al., 2012). In humans with ALS, EPHA4 expression inversely correlates with disease onset and survival, and loss of function mutations in *EPHA4* are associated with long survival (Van Hoecke et al., 2012). Expression of mutant *TARDBP*, encoding TDP-43, a protein found in neuronal inclusion bodies in patients with ALS and frontotemporal dementia, or knockdown with an AMO, induced motor neuron axonopathy in zebrafish embryos (Kabashi et al., 2010b; Laird et al., 2010). Simultaneous overexpression of human WT *TARDBP* mRNA *versus* mRNAs carrying the mutations showed that the WT mRNA may rescue the phenotype, whereas the mutant mRNAs may not (Kabashi et al., 2010b). Expression of WT human TARDBP alone did not cause a phenotype at the levels used, *e.g.* to rescue knockdown. This is important because WT TARDBP induced a phenotype on its own in other models, *i.e.* worm, fly and mouse, but not in zebrafish, emphasizing the greater appropriateness of this model. However, a recent experiment using knockout loss-of-function *tardbp* stable mutants demonstrated no phenotype as a result of compensation by the generation of a novel splice form of the duplicated *tardbpl* gene (Hewamadduma et al., 2013). Double-homozygous null mutants of *tardbp* and *tardbpl* had severe developmental abnormalities, including impaired spinal MN axon outgrowth (Schmid et al., 2013). If ALS is due to haploinsufficiency, then the AMO may model this better than compensation by *tardbpl* and complete loss of function of both genes. Progranulin, a neurotrophic factor, with mutations involved in frontotemporal lobar degeneration, is required for normal axonal outgrowth in zebrafish embryos. Knockdown of zebrafish progranulin genes induced MN axonopathy, prevented by overexpression of human *GRN* mRNA (Chitramuthu et al., 2010; Laird et al., 2010). Co-expression of human progranulin rescued the axonopathy induced by mutant *TARDBP* mRNA in zebrafish embryos, but had no effect on the mutant SOD1-induced phenotype (Laird et al., 2010). Similarly to TARDBP, FUS is an RNA binding protein which harbors aggregation-promoting domains (Li et al., 2013). Knockdown of zebrafish *fus* or overexpression of some human *FUS* alleles on their own yielded a motor phenotype that was rescued on co-expression of WT human *FUS* but not with ALS-related FUS mutations (Kabashi et al., 2011b). Knockdown and *FUS* RNA overexpression promoted abnormal NMJ structure and function (Armstrong and Drapeau, 2013a). WT human *FUS* rescued the *tardbp* knockdown phenotype, suggesting a common pathogenic pathway, while a lack of genetic interaction between *FUS*/*TARDBP* and *SOD1* was demonstrated (Kabashi et al., 2011b). However, co-expression of mutant *SOD1* and mutant FUS exacerbated the motor phenotype caused by overexpression of mutant *SOD1* alone (Kabashi et al., 2011b). Pharmacologically enhanced calcium entry with calcium channel agonists prevents the NMJ phenotype induced by an ALS human *TARDBP* mutation in zebrafish larvae (Armstrong and Drapeau, 2013b). A few other zebrafish genes have been investigated for the contribution of their loss of function in ALS-related symptoms. *Vapb* knockdown led to swimming deficits and the phenotype was rescued by overexpression of WT human *VAPB* mRNA, but not by overexpression of a VAPB mutant (Kabashi et al., 2013). Disruption in RNA processing is an element in the pathophysiology of several MN disorders and neurodegenerative diseases. Variants of the RNA polymerase II component, *ELP3*, are associated with an increased risk in ALS patients and knockdown in zebrafish embryos resulted

in motor axonal abnormalities, which may be disease related (Simpson et al., 2009). AMO-mediated knockdown of zebrafish *als2* induced severe developmental abnormalities, swimming deficits and abnormal MN projections (Gros-Louis et al., 2008) that may be related to the strongly induced EMS.

5.2. Upper motor neuron degeneration

5.2.1. Hereditary spastic paraplegias

HSPs, also known as familial Strümpell-Lorrain paraplegias, are a large group of heterogeneous inherited neurological disorders, characterized by progressive, usually severe, spasticity and pyramidal weakness, predominantly in the lower limbs (Depienne et al., 2007; Salinas et al., 2008; Stevanin et al., 2008).

The initial symptoms are usually stumbling and tripping due to a gait disturbance and stiffness in the legs (Stevanin et al., 2008). The disease is slowly progressive; most subjects display a markedly spastic gait and require a cane, walker, or wheelchair to move. Most patients share the same main features, including spastic gait, lower-limb hypertonia, hyperreflexia, extensor-plantar responses, muscle weakness, and bladder dysfunction, sometimes associated with decreased sensitivity to vibration at the ankles, and pes cavus or scoliosis. When these signs are isolated, the disease is described as "pure" or "uncomplicated." In "complicated" or "complex" forms, additional neurological or extraneurological features are observed, including mental retardation, peripheral neuropathy, cerebellar ataxia, epilepsy, brain magnetic resonance imaging anomalies, optic atrophy, retinitis pigmentosa, deafness, and cataract (Table 3).

Very few neuropathological studies have been performed (Stevanin et al., 2008). They have revealed dying-back axonal degeneration preceding cell-body degeneration in the longest fibers of the corticospinal tracts and dorsal columns, sometimes associated with loss of cortical neurons and anterior horn cells (Behan and Maia, 1974; Deluca et al., 2004). The fibers innervating the lower extremities are the first to be affected, suggesting that axonal transport, intracellular trafficking, and/or the maintenance of long fibers are specifically impaired in HSP. This hypothesis was recently corroborated by the identification of genes involved in these functions, together with the analysis of animal models. Indeed, several causative genes (>50) are currently known and insight into the functions of the proteins they encode have suggested that, in most cases, intracellular trafficking is affected by the deterioration of the ER, Golgi apparatus, endosomes, or axonal transport (Table 2). In addition, myelination, mitochondrial function, lipid metabolism, protein folding, and axon guidance may also be disrupted (Depienne et al., 2007; Fink, 2013; Finsterer et al., 2012; Goizet et al., 2009, 2011; Stevanin et al., 2008).

The stretch reflex is a muscle contraction in response to stretching within the muscle. This spinal reflex involves a monosynaptic pathway providing automatic regulation of muscle length. Type Ia afferent fibers from muscle spindles of one muscle make excitatory connections not only with the α-MNs innervating the same muscle but also with those innervating the synergic muscles. Moreover, *via* type Ia inhibitory interneurons, type Ia afferent fibers inhibit antagonistic muscles. When a muscle is stretched, type Ia afferent neurons increase their firing rate, leading to the contraction of the same muscle and its synergics and the relaxation of the antagonist, in a reaction known as reciprocal innervation. Type Ia inhibitory interneurons involved in the stretch reflex also receive inputs from the collateral fibers of some axons of the corticospinal tracts, making direct excitatory connections with spinal α-MNs. In this way, type Ia inhibitory interneurons are used to coordinate muscle contraction during voluntary movements. Axons from some brainstem descending tracts also have excitatory and inhibitory connections to this interneuron. Thus, the stretch reflex is mediated by multiple pathways acting in parallel *via* spinal and supraspinal pathways. Reflex responses mediated *via* supraspinal structures are known as "long-loop reflexes". While subcortical reflex pathways may be responsible for the afferent regulation of proximal muscle contractions, cortical projections may be of primary importance in modulating distal muscle contractions. Lesions in the supraspinal structures involved in long-loop reflexes may result in either hyperactivity or hypoactivity of the stretch reflexes.

The stretch reflex feedback loop provides the higher centers of the nervous system with a mechanism for adjusting muscle tone under different circumstances. Muscle tone disorders are frequently associated with lesions in the supraspinal descending pathways controlling the stretch reflex. Spasticity is a muscle tone disorder characterized by hyperactive tendon jerks and an increase in resistance to rapid muscle stretch. Partial denervation of the spinal neurons involved in the stretch reflex, inhibitory interneurons, and α- and γ-MNs leads to a gradual increase in sensitivity of these neurons. The synaptic contacts of UMNs are replaced by synapses with spinal cord neurons, resulting in a gradual increase in the influence of the reflexes on α-MN activity, reflected by the appearance of hyperreflexia. The increased influence of the muscle spindle results in massive contraction on stretching. Nevertheless, the muscle spindle response is mainly phasic, so when the muscle is stretched slowly or continuously, its activity decreases and the influence of the Golgi tendon organ becomes dominant, inhibiting the muscle contraction *via* the IIa inhibitory interneuron.

Key differences in human and zebrafish neuroanatomy of the cortical descending pathways preclude the use of zebrafish as a homologous model for UMN diseases, such as HSP. Nevertheless, zebrafish still represent a suitable partial model for a molecular and genetic dissection of the mechanisms involved in UMN disorders. Proprioceptors like the muscle spindle are not found in zebrafish myotomal muscle (Drapeau et al., 2002). Accordingly, the stretch reflex does not exist in zebrafish. The absence of both the corticospinal tract and stretch reflex preclude the possibility of reproducing the spasticity *per se*, the key diagnostic clinical finding of UMNs disorders in humans, in zebrafish. In fact, the spastic-like phenotypes described in some mutants (*tnt*, *roc*) or after treatment with strychnine are not really related to spasticity *sensu stricto*, but to other types of motor overactivity (Granato et al., 1996; Hirata et al., 2010). It is well established that strychnine induces motor overactivity by blocking the glycine function and a similar mechanism has been proposed for these two spastic-like mutants (Granato et al., 1996).

A functional analysis helps to demonstrate the causal relationship between defect and mutation. An identified HSP gene mutation in humans has been shown to induce pathogenic mechanisms in zebrafish, including MN axon defects, sometimes accompanied by locomotor impairment. Knockdown of *atl1* (SPG3A, atlastin) caused an abnormal MN axon architecture, correlated with a decrease in larval mobility, and genetic or pharmacological inhibition of the BMP signaling pathway rescued the induced phenotype (Fassier et al., 2010). Knockdown of *spast* (SPG4, spastin) inhibited spinal MN axon outgrowth and reduced axonal microtubule networks, causing extensive CNS-specific apoptosis and motility defects (Wood et al., 2006; Allison et al., 2013). Axon outgrowth defects were exacerbated by immersion of *spast*-morphant embryos in embryo medium containing the microtubule-destabilizing drug nocodazole (Butler et al., 2010). Simultaneous knockdown of *katna1*, encoding katanin p60, also involved in microtubule dynamics, and *spast*, exacerbated defects in spinal MN axon outgrowth (Butler et al., 2010). Protrudin (*ZFYVE27*, zinc finger, FYVE domain containing 27) may interact with spastin to modulate neurite outgrowth, as suggested by the

Table 3
Main genes involved in HSP determinism and available published data for modeling the pathology in zebrafish (for published data on other zebrafish HSP genes, see text).

Gene (protein)	Sub-type (OMIM)	Locus	Functional consequences	Frequency and/or number of families	Age at onset (years)	Type of spastic paraplegia and associated features	Ze
AD-HSP							
ATL1 (Atlastin-1)	SPG3A (182600)	14q22.1	GTPase, trafficking ER-Golgi, spastin and REEP1 partner	10% (39% in patients with early-onset)	Infancy (essentially <10 years)	Essentially pure HSP with rare peripheral neuropathy, incomplete penetrance	1
SPAST (Spastin)	SPG4 (182601)	2p22.3	Microtubule dynamics, endosomal trafficking, atlastin-1 and REEP1 partner	40% (12–18% in sporadic cases)	Variable	Essentially pure HSP with rare peripheral neuropathy or cognitive troubles, incomplete penetrance	2–5
KIF5A (kinesin 5A)	SPG10 (604187)	12q13.3	Axonal trafficking	2–3%	2–51	Pure or complicated HSP with peripheral neuropathy, parkinsonism	NA
BSCL2 (Seipin)	SPG17 (270685)	11q12.3	Protein located in the membrane of ER	Rare	Variable	*Silver syndrome*: distal amyotrophy of UL, allelic with CMT4D	NA
REEP1 (REEP1)	SPG31 (610250)	2p11.2	Protein located to mitochondria and ER chaperon, spastin and atlastin-1 partner	2–6.5%	Variable	Pure +/- white matter hyperintensities, incomplete penetrance	NA
KIAA0196 (Strumpellin)	SPG8 (603563)	8q24.13	Protein is part of the WASH complex, acting at the interface between actin regulation and endosomal membrane dynamics	Rare	Adult	Pure with associated UL involvement	6
AR-HSP							
CYP7B1 (CYP7B1)	SPG5 (270800)	8q12.3	Cholesterol et neurosteroid metabolism	7% (1–3% in sporadic cases)	1–40	Pure or complicated HSP with cerebellar ataxia, white matter hyperintensities	NA
PGN (Paraplegin)	SPG7 (607259)	16q24.3	Mitochondrial ATPase	8–21% (up to 7% in sporadic cases)	8–42	Pure or complicated HSP with peripheral neuropathy, optic atrophy, cerebellar atrophy +/- cerebellar ataxia	NA
KIAA1840 (Spatacsin)	SPG11 (604360)	15q21.1	Endosomal trafficking	21–26% (42–78% of HSP with TCC or ID)	1–30	Cognitive Tr or ID, peripheral neuropathy, RP (*Kjellin syndrome*), cerebellar ataxia, TCC and cerebellar atrophy, white matter hyperintensities, allelic with ALS5	7,8
ZFYVE26 (Spastizin)	SPG15 (270700)	14q24.1	Endosomal trafficking, mitochondrial dysfunction	2–5% (5–11% of HSP with TCC or ID)	4–19	Cognitive Tr or ID, peripheral neuropathy, RP (*Kjellin syndrome*), cerebellar ataxia, TCC and cerebellar atrophy, white matter hyperintensities	7
XL-HSP							
L1CAM	SPG1 (303350)	Xq28	Adhesin, neuritic growth, myelination	Very rare	Infancy	Complicated HSP (*MASA et CRASH syndromes*)	9
PLP1/proteolipid protein 1	SPG2 (312920)	Xq22.2	Myelin component	Very rare	1–18	Pure or complicated HSP (allelic with *Pelizaeus-Merzbacher disease*)	10

Abbreviations: AD, autosomal dominant; ALS, amyotrophic lateral sclerosis; AR, autosomal recessive; CMT, Charcot-Marie-Tooth, MASA syndrome, Mental retardation, Aphasia, Shuffling gait, Adducted thumbs; CRASH syndrome, Corpus callosum hypoplasia, Retardation, Adducted thumbs, Spastic paraparesis, and Hydrocephalus; ER, endoplasmic reticulum; ID, intellectual deficiency; L1CAM, L1 cell adhesion molecule; NA, not available; REEP1, receptor expression-enhancing protein 1; RP, retinitis pigmentosa; TCC, thin corpus callosum; Tr, troubles; UL, upper limbs; Ze, Zebrafish bibliographic references: [1]Fassier et al., 2010; [2]Allison et al., 2013; [3]Butler et al., 2010; [4]Wood et al., 2006; [5]Zhang et al., 2012; [6]Valdmanis et al., 2007; [7]Martin et al., 2012; [8]Southgate et al., 2010; [9]Wolman et al., 2007; [10]Brösamle, 2010.

rescue of double *spast*- and *zfyve27*-morphant embryos with co-injection of human WT spastin and protrudin (Zhang et al., 2012). Vacuolar protein sorting 37A (SPG53) is another protein involved in the endosomal sorting complex required for the transport system. A loss of motility was induced in *vps37a* knockdown AMO-injected zebrafish embryos without obvious dysmorphology, thus supporting the causal relationship between mutations in this gene and the spastic paraparesis described in patients (Zivony-Elboum et al., 2012). Knockdown of the zebrafish homolog of *KIAA0196* (SPG8, strumpellin) induced a curly-tail phenotype coupled with shorter motor axons. This phenotype was partially rescued by the human WT strumpellin but not the two mutants identified in HSP patients (Valdmanis et al., 2007). Similarly, AMO knockdown of *SLC33A1* (SPG42, solute carrier family 33 (acetyl-CoA transporter), member 1) in zebrafish embryos caused a curly-tail phenotype and defective axon outgrowth from the spinal cord. WT human

SLC33A1, but not a mutant form correlated with HSP, was able to rescue the morphant phenotype (Lin et al., 2008). However, the view that *SLC33A1*was equivalent to the SPG42 gene has been recently challenged (Huppke et al., 2012). The zebrafish is a powerful *in vivo* system for testing genetic interactions. Disruption of *spg11* (SPG11, spatacsin) or *zfyve26* (SPG15, spastizin) expression during zebrafish development induced a range of developmental defects, including locomotor impairment and abnormal architecture of spinal MN axons (Martin et al., 2012; Southgate et al., 2010). These two proteins are components of a multi-protein complex (Słabicki et al., 2010) and AMOs targeting the two genes at the same time suggested that they were involved in a pathway required for spinal MN axon outgrowth (Martin et al., 2012). However, an EMS (Raldúa et al., 2008) was apparently induced by changing the expression of these genes, thus making it difficult to interpret the phenotype, which may also be due to a developmental delay. Similarly, knockdown of *slc16a2* (SPG22, solute carrier family 16, member 2), the functional ortholog of human MCT8 thyroid hormone transporter (Arjona et al., 2011), caused developmental and neurological impairment (Vatine et al., 2013). Disturbance of the lipid metabolism is emerging as a subset of HSP types (Tesson et al., 2012). Some of the normal-looking *gba2* morphant embryos (SPG46, glucosidase, beta (bile acid) 2) exhibited abnormal locomotion and outgrowth of spinal MNs (Martin et al., 2013a,b). Mutations of *PNPLA6* (SPG39, patatin-like phospholipase domain containing 6) result in autosomal recessive HSP and this protein may also be one of the targets for distal damage to the longest axons induced by neuropathic organophosphorus compounds and chemical warfare agents (Glynn, 2013). Knockdown of the zebrafish homolog induced a strong developmental phenotype associated with impairment of MN axon development and an increase in axonal truncation and branching (Song et al., 2013). This phenotype was rescued by the introduction of WT, but not mutant, human *PNPLA6* mRNA. Knockdown modeling in zebrafish has been recently used for a functional validation of new candidate genes, *e.g. ARL6IP1* (SPG61, ADP-ribosylation factor-like 6 interacting protein 1), *PGAP1* (SPG67, Post-GPI attachment to protein 1), and *USP8* (SPG59, Unbiquitin specific peptidase 8) identified by whole-exome sequencing of HSP documented consanguineous human families (Novarino et al., 2014).

5.2.2. Primary lateral sclerosis

Primary lateral sclerosis (PLS) [OMIM#611637] is a pure UMN syndrome, characterized by progressive UMN degeneration with limb and bulbar dysfunction, but no LMN features (Sathasivam, 2010). PLS is generally considered a rare presentation of ALS with pure UMN involvement (Kiernan et al., 2011). Whether PLS is a separate entity from the ALS variant, or simply a different manifestation of this MND, remains controversial.

Patients with PLS tend to present 5–10 years earlier than those with ALS, have less limb wasting and bulbar symptoms during the course of the disease, and survive 6–7 years longer (Gordon et al., 2006; Tartaglia et al., 2007). To date, no genetic causes have been associated with PLS and therefore no data are available on zebrafish.

5.3. Lower motor neuron degeneration

5.3.1. Spinal muscular atrophy

SMA is a clinically and genetically heterogeneous disease, characterized by the degeneration of α-MNs in the ventral horn of the spinal cord, leading to progressive atrophy of skeletal muscles and paralysis (D'Amico et al., 2011). The most frequent form is inherited as an autosomal recessive trait, resulting from mutations in *SMN1* encoding for survival of motor neuron 1 (Burghes and Beattie, 2009; Lefebvre et al., 1995), and represents the second most common fatal autosomal recessive disorder after cystic fibrosis, with an incidence of 1/6000–1/10,000 live births (D'Amico et al., 2011). In fact, the term "SMA" is now reserved for SMN1-related SMA, all other forms being exceptional. Severe generalized muscle weakness and atrophy, predominantly in proximal limb muscles, are the hallmark of the disease. The phenotype is classified into four grades of severity (SMA types 1, 2, 3, and 4), based on age of onset and motor function achieved (D'Amico et al., 2011; Zerres et al., 1997). SMA type 1 (Werdnig-Hoffmann disease) [OMIM#253300] is the earliest, most severe, and most common type. Onset is before 6 months of age: patients never sit up and generally do not survive beyond the first 2 years. SMA type 2 (intermediate form) [OMIM#253550] is characterized by onset between 7 and 18 months. Patients do not achieve the ability to walk independently and the course of the disease is severe. Patients with SMA type 3 (mild, Kugelberg-Welander disease) [OMIM#253400] display highly variable severity of the disease, with an age of onset after 18 months. Proximal muscular weakness occurs during infancy. Some patients require wheelchair assistance while others continue to walk in adulthood with only minor muscular weakness. SMA type 4 has been added to this classification to describe with adult onset and mild course, without any risk of respiratory and nutritional troubles. All types of SMA are caused by homozygous deletion or mutation of *SMN1* (Hamilton and Gillingwater, 2013). In humans, there are two survivals of motor neuron genes: telomeric *SMN1* and its centromeric homolog *SMN2*, caused by the intrachromosomal duplication of 5q13. A single C to T substitution at base pair position 840 in *SMN2* compared to *SMN1* results in the exclusion of exon 7 from approximately 85–90% of *SMN2* transcripts. About 95% of patients have a homozygous disruption of *SMN1* due to deletion or gene conversion of *SMN1* to *SMN2* (Feldkotter et al., 2002). About 3% of affected individuals are compound heterozygotes for deletion of one *SMN1* allele and intragenic mutations. Loss of *SMN1* is essential to the pathogenesis of SMA, while the severity of the disease is primarily related to the number of copies of *SMN2*.

Non-SMN1 or non-5q SMA (or SMA plus phenotypes) have progressively become increasingly important (Guillot et al., 2008). Some have been accepted as separate entities definitely not linked to chromosome 5q markers (Zerres and Rudnik-Schoneborn, 2003). Among them, spinal muscular atrophy with respiratory distress (SMARD 1) [OMIM#604320] is a very rare autosomal recessive disorder. The onset is very early, characterized by initial respiratory insufficiency due to diaphragmatic palsy, symmetrical distal muscular weakness, muscle atrophy, peripheral sensory neuropathy, and autonomic nerve dysfunction (Grohmann et al., 2003; Kaindl et al., 2008). The prognosis is poor with early death. Survival up to a few years has been reported only with assisted ventilation. The causative gene, *IGHMBP2*, encodes immunoglobulin μ-binding protein 2, located on chromosome 11q13 (Grohmann et al., 2001). Lethal congenital contracture syndrome 1 (LCCS1) [OMIM#253310] is an exceptional autosomal recessive condition with prenatal onset, leading to total immobility of the fetus, accompanied by hydrops, micrognatia, pulmonary hypoplasia, pterygia, and arthrogryposis (Makela-Bengs et al., 1998). It invariably leads to prenatal death before the 32nd gestational week. Neuropathological analysis reveals a lack of anterior horn motoneurons, severe atrophy of the ventral spinal cord, and hypoplastic, almost absent skeletal muscles (Herva et al., 1988). A milder phenotype, lethal arthrogryposis with anterior horn cell disease (LAAHD), is characterized by fetal akinesia, arthrogryposis, and MN loss, leading to early death following respiratory failure after the delivery (Nousiainen et al., 2008). Neuropathological findings in LAAHD resemble those of LCCS1, but are less severe. Mutations in the *GLE1* RNA export mediator are the cause of these

two allelic conditions (Nousiainen et al., 2008). Spinal muscular atrophy with progressive myoclonic epilepsy (SMA-PME) [OMIM#159950] is also extremely rare (Haliloglu et al., 2002; Jankovic and Rivera, 1979). Affected patients display severe, progressive myoclonic epilepsy and LMN disease, proven by electrophysiological and muscle-biopsy findings. The course of the disease is severe, leading to respiratory muscle involvement and severe handicap or death before 20 years of age. Mutations in the *ASAH1* gene have very recently been demonstrated to cause SMA-PME (Zhou et al., 2012). Mutations of the same gene are responsible for Farber disease, a very rare autosomal recessive condition resulting from a marked reduction in lysosomal acid-ceramidase activity (under 10%) or its total absence. Farber disease is characterized by early onset subcutaneous lipogranulomata, joint pain, and hoarseness of the voice. A higher residual acid-ceramidase activity might be responsible for SMA-PME. It should be pointed out that the distinction between developmental and degenerative conditions is becoming less and less clear in neurological conditions. Indeed SMA type 1, SMARD1, LCCS1, and SMA-PME have very early, sometimes prenatal, onset and are clearly related to an MN development disorder, but these abnormally developed MNs will ultimately degenerate. These clinical conditions are now included by all authors in MNDs.

PCH1 [OMIM#614678] is a distinctive subtype of pontocerebellar hypoplasia (PCH) only diagnosed in 13 unrelated families from around the world, characterized by congenital onset of diffuse muscle wasting secondary to spinal cord anterior horn cell loss and cerebellar hypoplasia (Wan et al., 2012). Neurogenic muscle atrophy with spinal motor neuron disease has been confirmed by electromyography, muscle biopsy, or autopsy. Exome sequencing has identified recessive mutations in *EXOSC3*, coding for exosome component 3, highlighting the implication of RNA processing dysregulation in the abnormal development and degeneration of cerebellar and spinal motor neurons (Wan et al., 2012).

Autosomal dominant SMA (AD-SMA), also known as dominant congenital SMA (DCSMA) or autosomal dominant lower extremity-predominant spinal muscular atrophy-2 (SMALED2) [OMIM#615290], is a very rare disorder of developing anterior horn cells, characterized by congenital onset with predominantly proximal lower-limb features and very slow progression throughout life (Peeters et al., 2013). Clinical overlaps with HSP and DCSMA with upper motor neuron have been highlighted in the 8 affected families reported to date (Oates et al., 2013; Neveling et al., 2013). In these families, heterozygous dominant mutations have been identified in *BICD2*, encoding bicaudal D homolog 2 (*Drosophila*), a key adaptor protein that interacts with the dynein–dynactin motor complex, which facilitates the intercellular trafficking critical to motor neuron development and maintenance (Oates et al., 2013).

SMA and other lower MNDs are clinically characterized by muscle atrophy and muscle weakness, probably resulting from skeletal muscle denervation (Nicole et al., 2002). Denervation, defined as an NMJ unoccupied by α-MNs, is common in lower MNDs. Thus, after a short presymptomatic period, mutant mice with an intermediate phenotype began to have difficulty ambulating and righting themselves. Histological analysis of skeletal muscle at 2 weeks revealed atrophy, with denervated neuromuscular junctions and reduced acetylcholine receptor (AChR) clusters (Lee et al., 2013). The extent of denervation generally correlates with the degree of severity in mouse SMA models (Kariya et al., 2008).

Zebrafish has been used as a vertebrate model of SMA by using AMO to reduce the survival of motor neuron protein levels or generating *smn* (*smn1*) mutants, although *smn* AMO exhibited defects in spinal LMN axon outgrowth and pathfinding (Carrel et al., 2006; McWhorter et al., 2003; Oprea et al., 2008; Winkler et al., 2005). The zebrafish phenotype was rescued by full length human SMN but not the mutated version (Carrel et al., 2006). When zebrafish *smn* morphant NMJs were analyzed at 3 dpf, both normal and aberrant axons colocalized with AChR clusters, suggesting that although *smn* AMO did not have the stereotyped pattern of innervations, the muscle fibers were innervated by axons (McWhorter et al., 2003). Similarly, the presynaptic marker synaptic vesicle protein 2 (SV2) colocalized with AChR clusters and no NMJ defects were observed in 9 dpf *smn* mutants with reduced levels of survival motor neuron protein (Boon et al., 2009). However, in 11 dpf *smn* mutants, a marked decrease in SV2 was found at the NMJ. Interestingly, other presynaptic markers, such as synaptotagmin II and synaptophysin, were unaffected (Boon et al., 2009). Decreased size and low activity has been described in these *smn* mutants. When mutants were generated lacking both maternal and zygotic SMN there was very low levels of SMM protein and the mutants display motor axon defects (Hao et al., 2013). This maternal:zygotic-*smm* zebrafish mutant has low levels of SMN due to the leaky heat shock promoter driving human SMN and thus is a true, severe genetic model of SMA. Conditional transgenesis revealed that SMN protein was needed during the whole MN morphogenesis process and to rescue motor axon defects and motor deficits on a zebrafish null *smn* mutant background (Hao et al., 2013). This suggests that an early developmental defect in MNs through a low level of SMN may have a long-term deleterious effect on connectivity and movement. A depressed actin-binding and bundling protein, plastin 3 (PLS3), level has also been found in *smn* mutants and this protein was able to rescue axon growth defects associated with low SMN in zebrafish (Hao et al., 2012; Oprea et al., 2008). This protein acts as a protective modifier of SMA in improving axon outgrowth, counteracting poor axonal connectivity at SMA NMJs (Ackermann et al., 2013; Oprea et al., 2008) through is Ca^{2+} binding residues (Lyon et al., 2014). Consequently, zebrafish represent a suitable model for finding SMN1 interacting partners able to modulate an SMN1-deficiency phenotype (Akten et al., 2011; Lotti et al., 2012). As it has been demonstrated that transgenic zebrafish expressing human *SMN2* rescued the neuromuscular presynaptic SV2 defect in *smm* mutants and increased their survival (Hao et al., 2011), a genetic model of SMA has been implemented in zebrafish. Novel candidate genes implicated in SMA phenotype have been identified in transcriptome analyses of SMN deficient zebrafish (See et al., 2014). Whole embryos rather than isolated motor neurons were used in this approach to take into consideration transcripts expressed in non-MN cell types potentially able to mediate non-cell autonomous effects. Neurexin2a was identified as a strongly down-regulated gene and the knockdown of *nrxn2a* resulted in motor CaP MN axon truncations and branching defects and phenocopies of SMN defects, including reduced motor axon excitability (See et al., 2014). The partial rescue of SMN morphants by *nrxn2ab* mRNA injection strongly suggested that *nrxn2a* acted as a downstream SMN mediator. Chondrolectin is a gene identified in a splicing array to be decreased in mouse SMN mutants and knockdown and overexpression in zebrafish embryos indicated a role in MNs axon maintenance as well as pathfinding (Zhong et al., 2012). MN axon defects caused by *smn* AMO can be rescued by *chodl* overexpression supporting chondrolectin as an *in vivo* genetic modifier of the SMA phenotype (Sleigh et al., 2014). The involvement of ubiquitin homeostasis and β-catenin signaling has been also explored by using a zebrafish model of SMA (Wishart et al., 2014).

Although muscle atrophy, measured as a decrease in muscle fiber size, is an easy endpoint to analyze in zebrafish (Cao et al., 2009; Hanai et al., 2007), to the best of our knowledge, no information is currently available on muscle atrophy in zebrafish models of SMA. Therefore, zebrafish is a very useful homologous

model for LMN diseases, although additional efforts should be made to analyze the clinical features of this group of diseases, muscle atrophy and weakness.

It should be pointed out that the zebrafish has been used as a functional validation test for deciphering the effect of rare human variants associated with LMN diseases. GLE1 depletion in zebrafish embryos induced an LCCS1-like phenotype and apoptosis of neural precursors leading to defective spinal MNs (Jao et al., 2012). AMO knockdown of *asah1b*, a homolog of human *ASAH1* associated with SMA-PME, led to a marked loss of MN axonal branching and a concomitant increase in apoptosis in the spinal cord (Zhou et al., 2012). AMO knockdown of *exosc3* in zebrafish embryos caused general embryo maldevelopment similar to EMS, resulting in small brain size and poor motility, reminiscent of some human clinical features of PCH1, and these defects were largely rescued by co-injection with human WT but not mutant *exosc3* mRNA (Wan et al., 2012).

5.3.2. Distal hereditary motor neuropathy, spinal Charcot-Marie-Tooth disease, or distal spinal muscular atrophy

Distal hereditary motor neuropathy (dHMN), also referred to as distal SMA or spinal CMT, represents a group of clinically and genetically heterogeneous diseases caused by degeneration of spinal motor neurons (Devic et al., 2012; Rossor et al., 2012). Spinal CMT is defined by a slowly progressive, symmetrical, predominantly distal lower motor neuron phenotype. Electroneuromyograms reveal pure motor neuropathy. Many forms of dHMN have minor sensory abnormalities and/or a significant upper-motor-neuron component, and there is often an overlap with the dominant axonal forms of CMT (CMT2), juvenile forms of ALS, and HSP. Eleven causative genes and four loci have been identified with autosomal dominant, recessive, and X-linked inheritance patterns (Table 4). The encoded proteins are implicated in protein misfolding, axonal transport, RNA metabolism, cation-channel function, and mitochondrial network maintenance mechanisms that overlap with other MND.

Modeling CMT2 in zebrafish by inducing mutations in *LRSAM1* produced a modified phenotype of *lrsm1* morphants associated with abnormal MN development (Weterman et al., 2012). Homozygous deletions of *Mfn2* in mice cause placental abnormalities and embryo death (Chen et al., 2003), thus precluding the use of this animal as a model for CMT2A2. AMO targeting a splice site of zebrafish *mfn2* showed severe, early developmental effects, including truncated MNs, fewer NMJs, and abnormal muscle fibers (Vettori et al., 2011). In contrast, *mfn2* null allele embryos initially appeared healthy but exhibited a progressive loss of motor function with defective axonal transport of mitochondria and alterations at the NMJ level (Chapman et al., 2013).

5.3.3. Spinal and bulbar muscular atrophy

Spinal and bulbar muscular atrophy (SBMA) [OMIM#313200], or Kennedy's disease, is an adult-onset disorder affecting both muscle and LMNs (Kennedy et al., 1968). Patients present amyotrophic, proximal or distal weakness and wasting of the facial, bulbar and limb muscles, as well as occasional sensory disruptions, associated with endocrine disturbances, including androgen resistance, gynecomastia, elevated testosterone or progesterone, and reduced fertility (Finsterer, 2009; Kennedy et al., 1968). The course is slowly progressive; the ability to walk lost only late in life; only few patients require ventilator support, and life expectancy is only slightly reduced. SBMA is an X-linked recessive polyglutamine disorder caused by expansion of a polymorphic CAG tandem-repeat in exon 1 of the androgen-receptor gene (La Spada et al., 1991). The cardinal histopathology findings are loss of anterior horn cells in the brain stem and spinal cord.

5.3.4. Other motor neuron syndromes

The Brown-Vialetto-Van Laere (BVVL) [OMIM#614707] and Fazio-Londe syndromes (FL) [OMIM#211500] are very rare motor neuron disorders with clinical overlap, as they share bulbar palsy and respiratory insufficiency (Bosch et al., 2012; Ciccolella et al., 2012). In BVVL, a sensorineural deafness is observed in addition to

Table 4
Main genes involved in dHMN determinism and available published data for modeling the pathology in zebrafish.

Gene	Locus	OMIM	Disease	Phenotype	In	Ze
HSPB1	7q11.23	*602195	dHMN type I	Childhood or juvenile onset of distal LL wasting and weakness	AD	NA
HSPB8	12q24	*608014				NA
GARS	7p15	*600287				NA
DYNC1H1	14q32	*600112				1
HSPB1	7q11.23	*602195	dHMN type II	Adult onset of distal LL wasting and weakness	AD	NA
HSPB8	12q24	*608014				NA
BSCL2	11q13	*606158				NA
HSPB3	5q11	*604624				NA
?	11q13		dHMN type III	Adult onset of distal LL wasting and weakness	AR	
?	11q13		dHMN type IV	Adult onset of distal LL wasting and weakness	AR	
GARS	7p15	*600287	dHMN type V	UL onset	AD	NA
BSCL2	11q13	*606158				NA
IGHMBP2	11q13	*600502	dHMN type VI	Infantile onset of distal wasting and weakness and respiratory failure	AR	NA
DCTN1	2p13	*601143	dHMN type VII	Adult onset of distal wasting and weakness and vocal-cord paralysis	AD	2
TRPV4	12q24	*605427				3
ATP7A	Xq12	*300011	X-linked dHMN	Variable onset of distal LL wasting and weakness	XL	NA
SETX	9q34	*608465	dHMN and pyramidal features	Distal LL wasting and weakness and pyramidal features	AD	NA
BSCL2	11q13	*606158				NA
TRPV4	12q24	*605427	Congenital dHMN	Distal weakness at birth and arthrogryphosis	AD	3

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; In, inheritance; LL, lower limbs; NA, not available; UL, upper limbs; XL, X-linked. Ze, Zebrafish bibliographic references: [1]Langworthy and Appel, 2012; [2]Del Bene et al., 2008; [3]Amato et al., 2012.

other features, but BVVL and FL are considered as the same entities. The age at onset is highly variable, ranging from infancy to adulthood. Classically, a sensorineural hearing loss is the sign at onset in BVVL, followed by other lower cranial nerve involvement and lower motor neuron symptoms in the limbs (Spagnoli and De Sousa, 2012). Upper motor neuron symptoms are less frequently described. In FL, earlier onset is generally associated a more rapidly progressive course, leading to respiratory distress, and weakness in the face and all four limbs, but mainly the upper limbs. BVVL and FL are caused by riboflavin deficiency secondary to mutations in one of the following three genes: *SLC52A3*, *SLC52A2* and *SLC52A1*, each them coding for a riboflavin transporter. Oral riboflavin supplementation seems to stabilize and improve progression of the disease.

Triple A syndrome (3A syndrome or Allgrove syndrome) [OMIM#231550], is a rare autosomal recessive disorder characterized by adrenocorticotropic hormone resistant adrenal deficiency, achalasia of the esophageal cardia, and alacrimia (Allgrove et al., 1978). Autonomic and neurological dysfunction, including UMNs and LMNs involvement, frequently complete the clinical spectrum (Goizet et al., 2002; Houlden et al., 2002; Messina et al., 2009). 3A syndrome is caused by mutations in *AAAS* gene coding for ALADIN, a ubiquitous protein containing four WD repeats, aimed to form a typical β-propeller structure (Tullio-Pelet et al., 2000). WD repeats typically occur multiple times within a protein. These domains are involved in protein–protein interactions that may act in signal transduction, intracellular trafficking, cytoskeleton assembly, transcription, and cell division control (Smith et al., 1999). There are currently no data on modeling SBMA, BVVL, FL, and 3A syndrome in zebrafish but this animal may be used to dissect the molecular mechanisms involved.

6. Some future prospects

Recent technological advances and intrinsic qualities of the zebrafish, *e.g.* rapid, external development and optical observation both early and late organogenesis stages, using adult transparent *casper* or *pinky* lines (Hsu et al., 2013; White et al., 2008), make this experimental model of major significance for biomedical research in general and MNDs in particular. Techniques including labeling different cell types and distinct subcellular compartments in live fluorescent transgenic reporters, the use of *in vivo* two-photon microscopy, laser ablation, Ca^{2+} imaging, and patch clamp electrophysiology, even in the embryonic and larval stages, has made it possible to characterize the *in vivo* dynamics of processes in neurons and neural networks and early neurological changes in diseases. For example, the transgenic *mitofish* line has made it possible to study *in vivo* mitochondrial distribution and transport in Rohon-Beard sensory neurons, as well as some induced defects in a tauopathy model (Plucińska et al., 2012). This approach may be combined with vital dyes for cellular, subcellular, and functional imaging (Romanelli et al., 2013). New *in vivo* non-invasive technologies, including conditional transgenesis (Hans et al., 2011) and optogenetics (Arrenberg and Driever, 2013; McLean and Fetcho, 2011) are emerging for monitoring the temporal requirements of MN development and survival, as well as motor neuron activities. This has recently been demonstrated in the case of the requirement of SMN1 for proper MN morphology in embryonic stages with long-term effects (Hao et al., 2013) and to identify brain (Ahrens et al., 2013; Sumbre and Poo, 2013) and spinal cord (Fajardo et al., 2013; Fidelin and Wyart, 2014; Kimura et al., 2013; Wyart et al., 2009) neurons critically involved in locomotor drive. These approaches may be used for *in vivo* imaging and monitoring the dynamics of the appearance of neurodegenerative disease-related biological processes.

Zebrafish have a very interesting potential for testing whether mutations that represent risk factors with low penetrance and/or that generate compounds toxic to neurons and accumulate over time may cause MNDs to be more deleterious with additional genetic variants and/or combine with environmental factors that enhance the risk. The knockdown of *epha4* or its pharmacological inhibition using 2,5-dimethylpyrrolyl benzoic acid rescued the axonopathy induced by mutant SOD1 and TARDBP expression, as well as the motor axon phenotype induced by knockdown of *smm1* (Van Hoecke et al., 2012). The excitotoxic effects of calcium are well described for late stages of ALS manifestation, *i.e.* leading to cell death, but the opposite may be true at earlier, pre-clinical stages which are the most important for drug development. Calcium channel agonists protect against neuromuscular dysfunction in a zebrafish model of *TARDBP* mutation (Armstrong and Drapeau, 2013b). In addition, the antiexcitotoxic drug riluzole, that inhibits neuronal sodium channels in zebrafish (Tong and McDearmid, 2012) and is an approved drug for use in ALS, and apomorphine, known to be an activator of the NRF2 antioxidant response, were able to reduce the neuronal stress in a *sod1* zebrafish model of ALS (Da Costa et al., 2014; McGown et al., 2013). These findings demonstrated the potential of this model to identify modifier genes and chemical suppressors of disease phenotypes that generically modulate the vulnerability of neurons to degeneration. The ease of performing whole-organism, *in vivo* bioassays of zebrafish offers an unparalleled opportunity for understanding how genes and their variants interact with exposure to environmental toxicants and drugs, either to preserve health or cause disease. This good predictive model may be relevant for MNDs with a high incidence of sporadic cases, such as ALS (Chang and Wu, 2009; Trojsi et al., 2013).

In conclusion, genetic analyses in humans together with *in vitro* and *in vivo* studies using model systems, like mice and zebrafish, have greatly expanded our knowledge of MNDs. As described in this review, the zebrafish has been used to study molecular and functional aspects of some MNDs in an *in vivo* context. However, this type of research is still in its infancy: relatively few human mutations have been investigated to date and there is great potential for combining different approaches to discover new genetic and environmental factors, alone or in combination. The model also offers very promising avenues for deciphering the *in vivo* deteriorations of physiological processes underlying unexplored MNDs.

Conflict of interest

None.

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